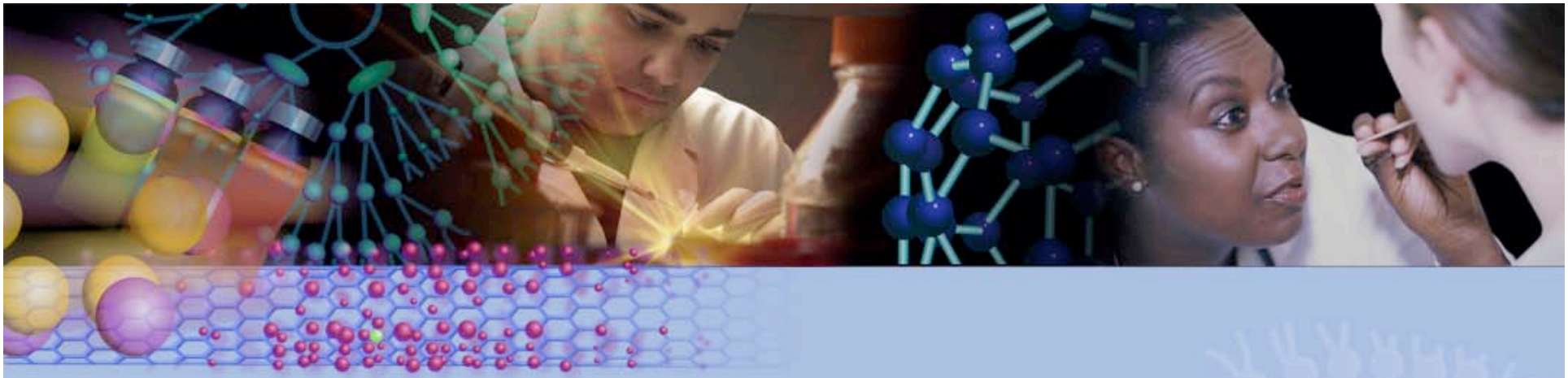


Developing In Vitro Tools and Models for Understanding Nanotoxicology

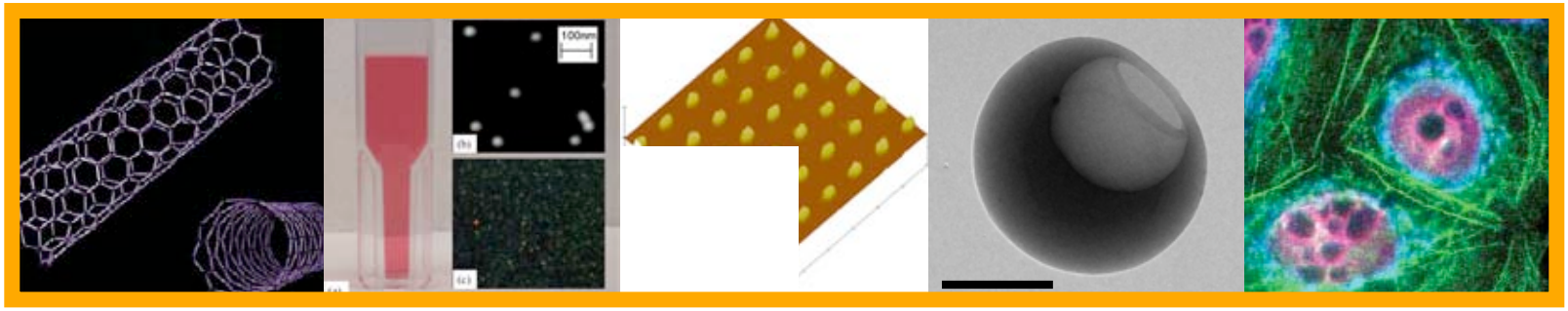
Life Science Division

Lawrence Berkeley National Laboratory

Human interaction with nano

Nano – smaller and better

Nanomaterial is matter at dimensions of roughly 1~100 nm, where unique phenomena enable novel applications



- Optical, electromagnetic, mechanical enhancement
- Increasing stability or reactivity, smaller size, higher surface/mass ratio

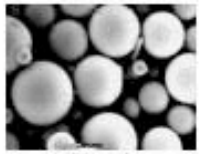
The Scale of Things – Nanometers and More

Things Natural

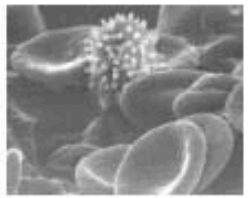
Dust mite
200 µm

Ant
~ 5 mm

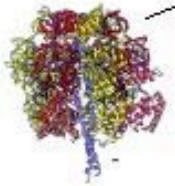
Human hair
~ 60-120 µm wide



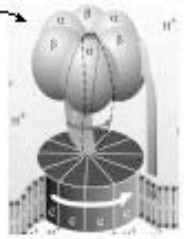
Fly ash
~ 10-20 µm



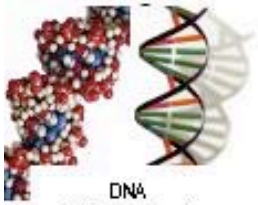
Red blood cells
with white cell
~ 2-5 µm



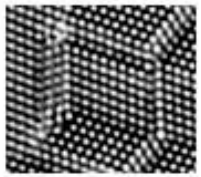
~10 nm diameter



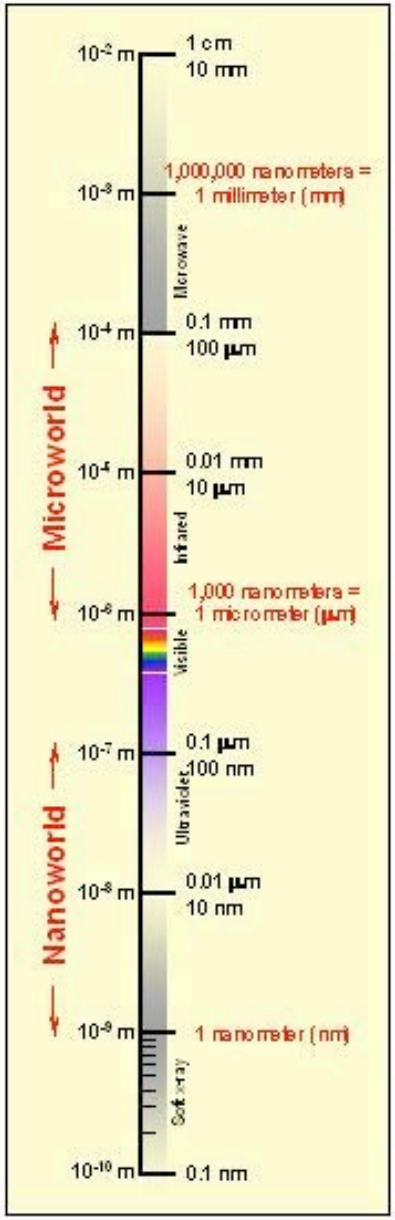
ATPsynthase



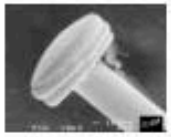
DNA
~2-12 nm diameter



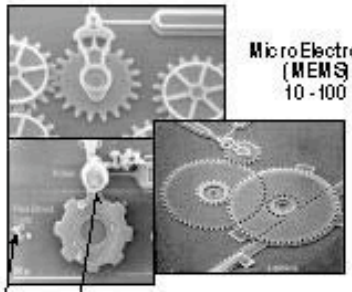
Atoms of silicon
spacing ~tenths of nm



Things Manmade

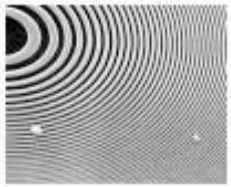


Head of a pin
1-2 mm

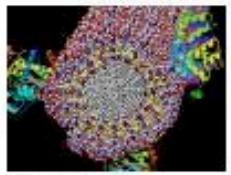


MicroElectroMechanical
(MEMS) devices
10 - 100 µm wide

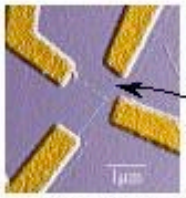
Pollen grain
Red blood cells



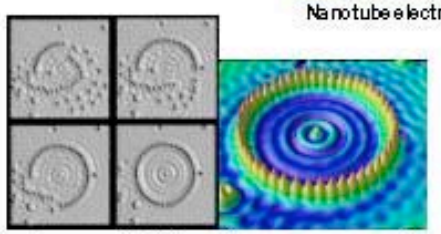
Zone plate x-ray "lens"
Outer ring spacing ~35 nm



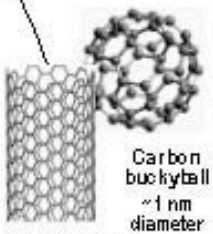
Self-assembled,
Nature-inspired structure
Many 10s of nm



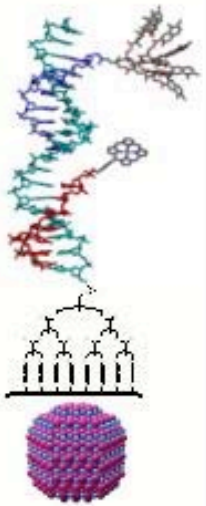
Nanotube electrode



Quantum corral of 48 iron atoms on copper surface
positioned one at a time with an STM tip
Corral diameter 14nm



The Challenge

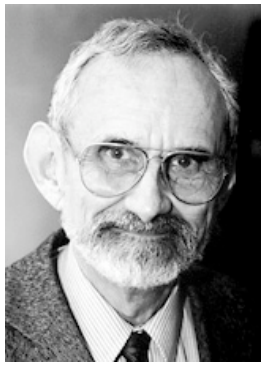
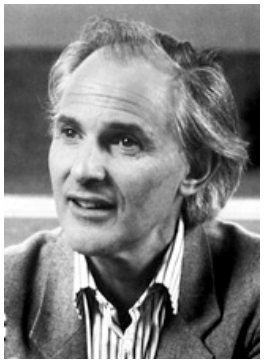
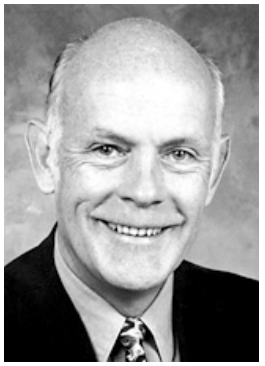


Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.

BES, DOE



Nobel Prize, Phisics 1986
Ernst Ruska, Heinrich Rohrer, and Gerd Binnig

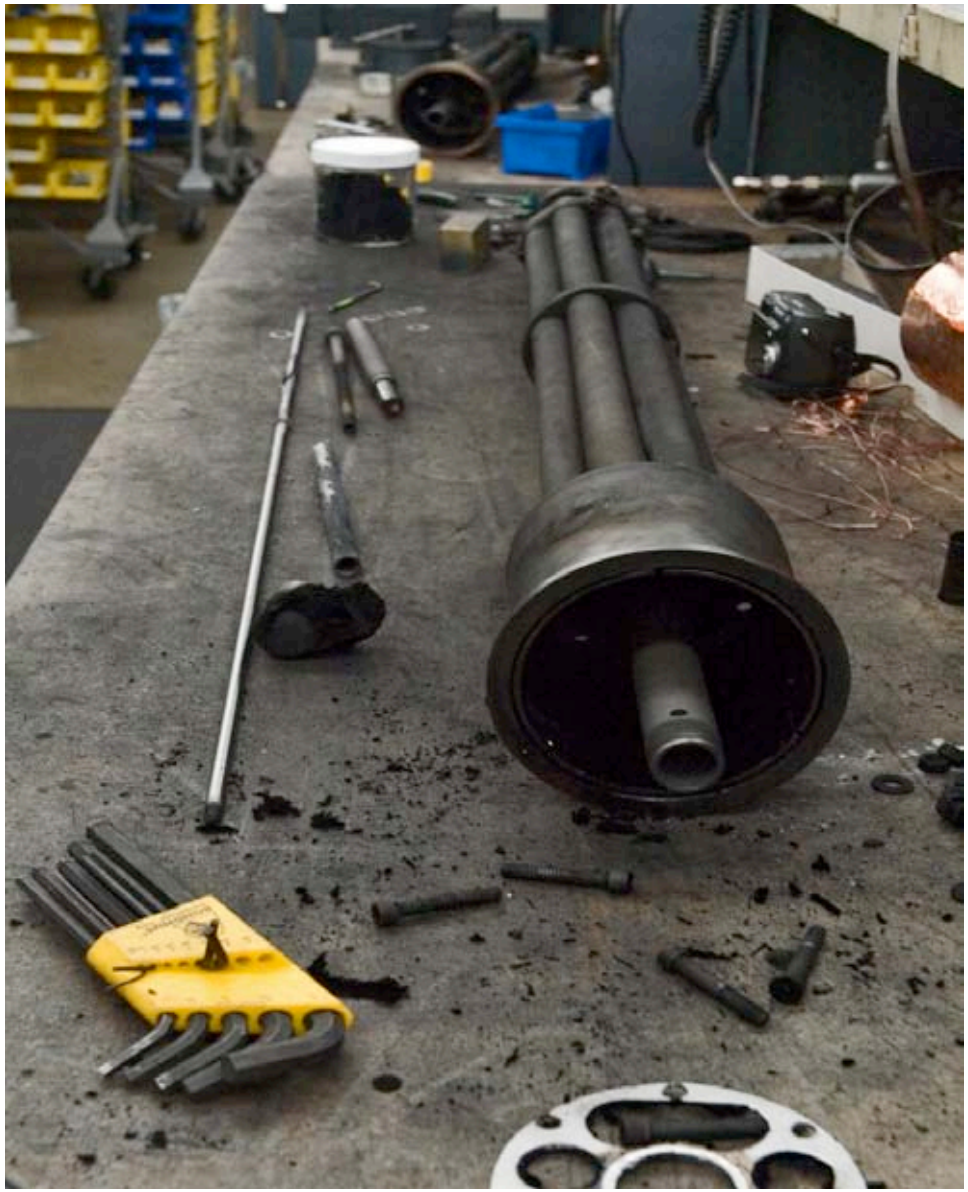




Nobel Prize, Chemistry, 1996
Robert F. Curl Jr., Sir Harold W. Kroto, and Richard E. Smalley

Nanotechnology is the new wave of technology innovation for the 21st century.

As nanoscience and nanotechnology come of age, the time for actively addressing the hazards associated with nanomaterials has arrived.

- Barnard AS. *Nature Materials* , 2006



Courtesy, Monteiro, EMS 2007

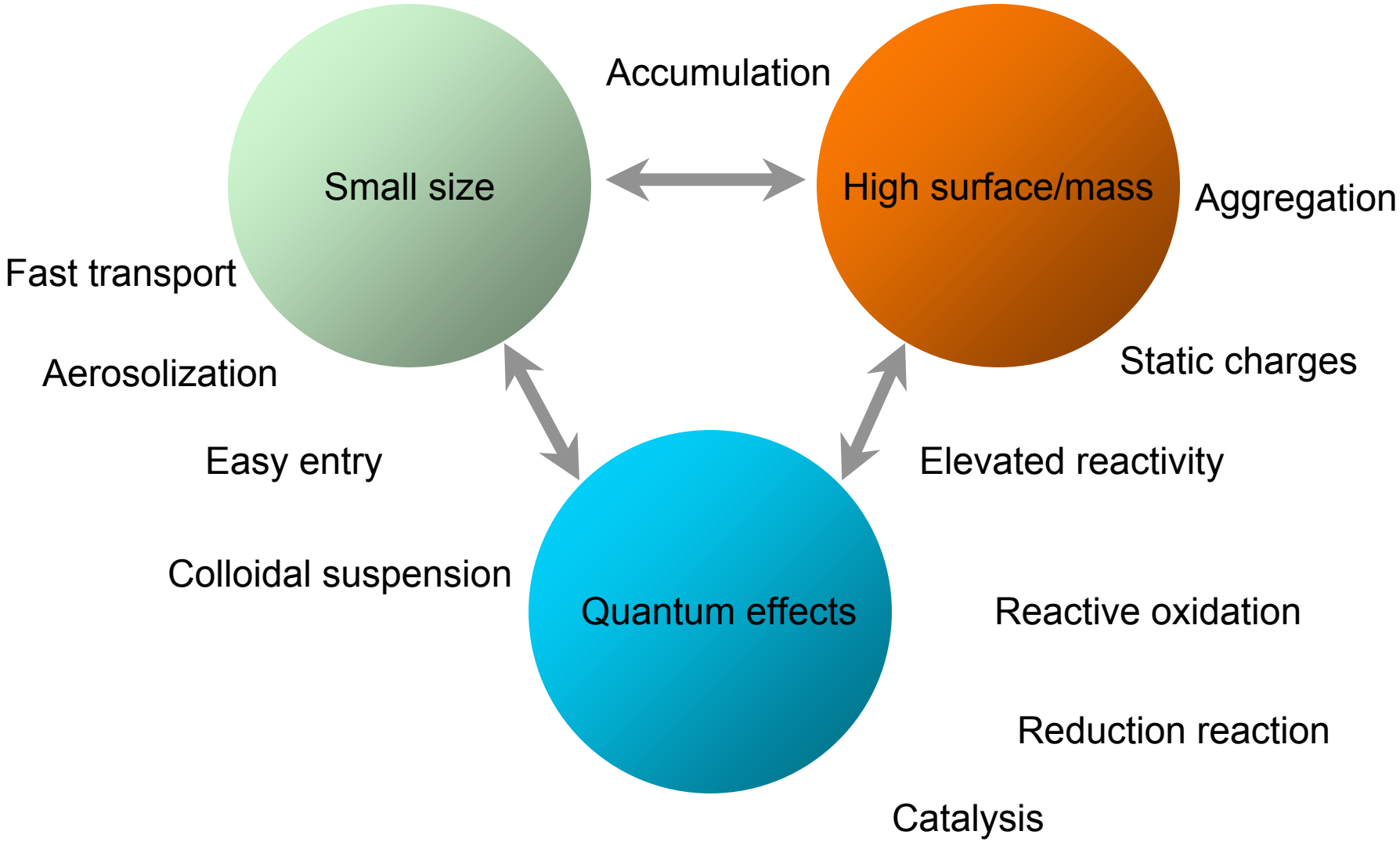
Is that a nanocapsule on your face?

smalltimes
BIG NEWS IN SMALL TECH.

- **Day 1:** I can't help but feel just the slightest bit invaded.
- **Day 2:** My eyelids itch… Are the nanocapsules going to penetrate clear into my eyeballs?
- **Day 3**: My eyelids still itch. The marketing folks at L'Oreal must understand how [nanocapsules] could freak people out.
- **Day 4:** My eyelids don't itch anymore. But now my cheeks are starting to burn.
- **Day 5:** I used my regular old non-nanocapsulated moisturizer today. Call me a wimp, but I needed a break from this strange stuff.

Article URL: http://www.smalltimes.com/document_display.cfm?document_id=7554

Physico-chemical properties of nanomaterial



What is different: Nanoparticles vs Larger Particles *(respiratory tract as portal-of-entry)*

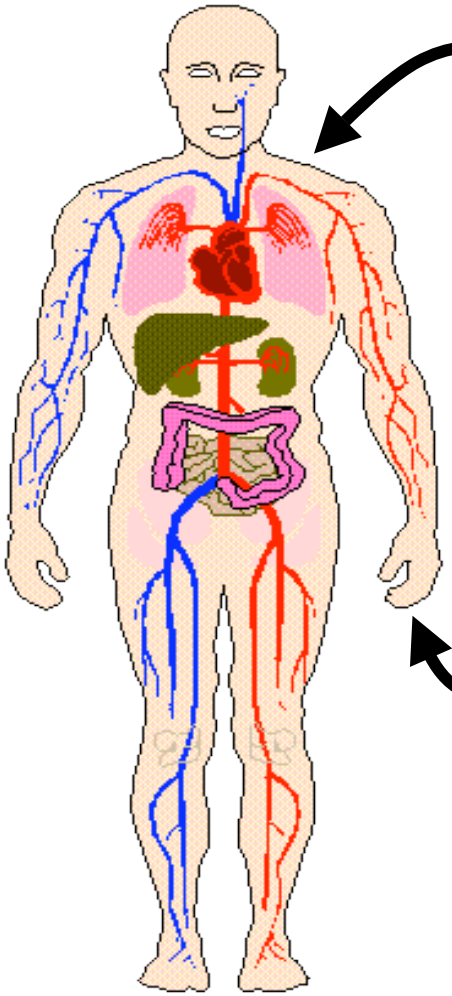
	Nanoparticles (<100 nm)	Larger Particles (>500 nm)
Physico-chemical characteristics:		
Ratio: number/surface/area/volume	high	low
Agglomeration	likely (dependent on medium; surface)	less likely
Deposition	diffusion; throughout resp. tract	sedimentation, impaction, interception; throughout resp. tract
Protein/lipid adsorption	very effective and important for biokinetics and effects	less effective
Translocation to secondary target organs:	yes	generally not (to liver under "overload")
Clearance		
— mucociliary	probably yes	efficient
— alv. macrophages	poor	efficient
— epithelial cells	yes	mainly under overload
— lymphatic	yes	under overload
— blood circulation	yes	no
— sensory neurons (uptake + transport)	yes	no
Cell entry:	yes (caveolae; clathrin; lip. rafts; diffusion)	yes (diff. mechanisms)
— mitochondria	yes	no
— nucleus	yes (<40 nm)	no
Effects *(caveat: dose!)* :		
— inflammation	yes	yes
— oxidative stress	yes	yes
— activation of signaling pathways	yes	yes
— genotoxicity, carcinogenicity	?	some

Oberdorster, EMS, 2007

Potential Health Hazards

- Extensive use of nanotechnology in biotech, pharmaceutical, chemical, and high-tech industries
- Solubilization, biocompatibilization, surface coating modifications
- Long-term persistence/structure stability
- Fast *in vivo* transportation
- Bioaccumulation
- Multiple entry routes, e.g. food (fish, plants, etc.), water, air entry routes)
- Cellular effects (stress responses, carcinogenesis, mutagenesis, cell cycle, cell death, differentiation, extracellular matrix, inflammation, DNA damage)

Cross-cutting issue: Size and Translocation



Dogma (Oberdorster, 2004):

- D < 100 nm can translocate into brain
- D < 100 nm has impact on CNS

Challenges (Warheit, 2006):

- Composition trumps size for effects

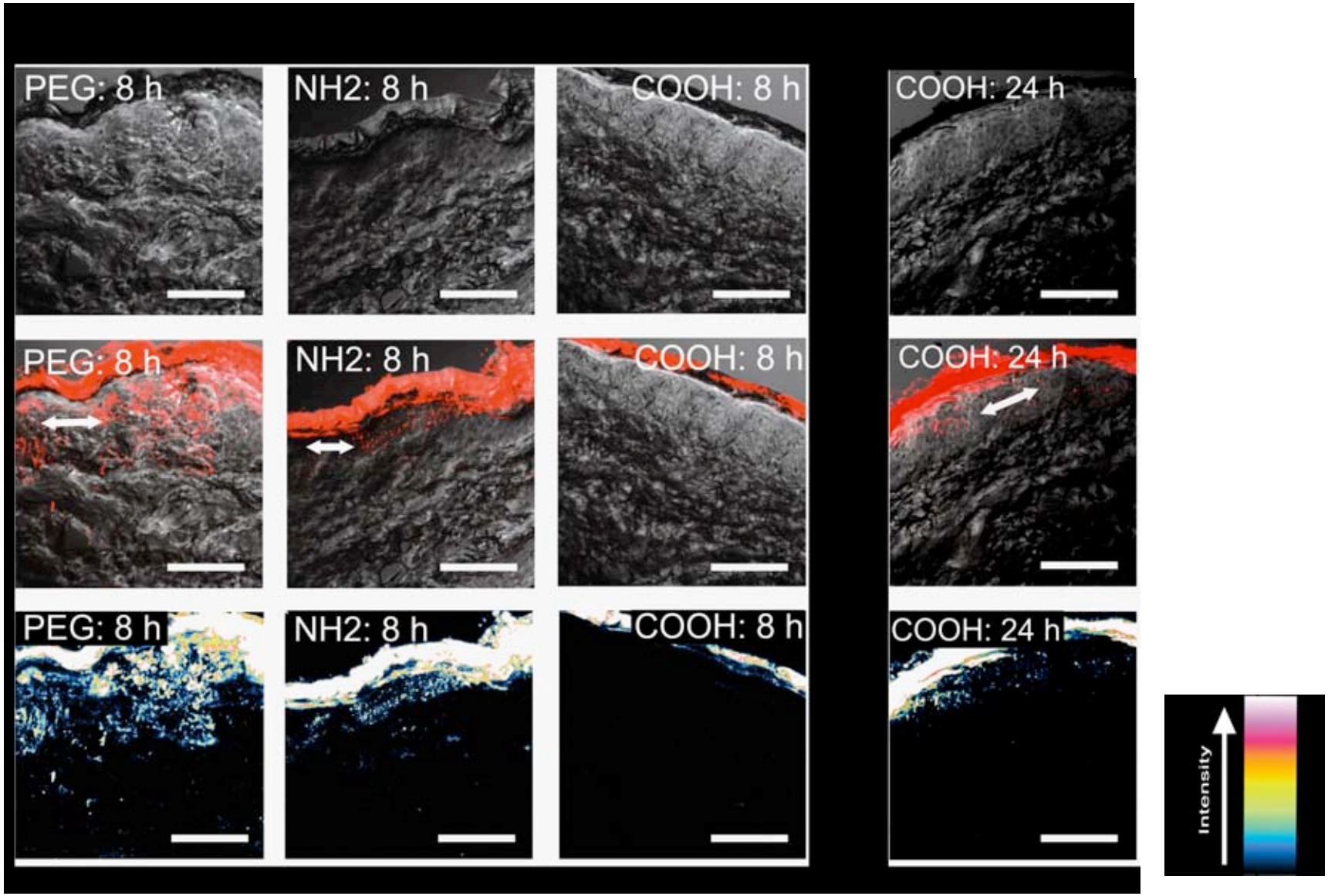
Dogma:

- Skin is a tight barrier
- Hydrophobic materials transport better

Challenges (Tinkle, Montiere-Riviere 2005-6)

- Particles into dermis of stressed skin
- Quantum dots found in dermis

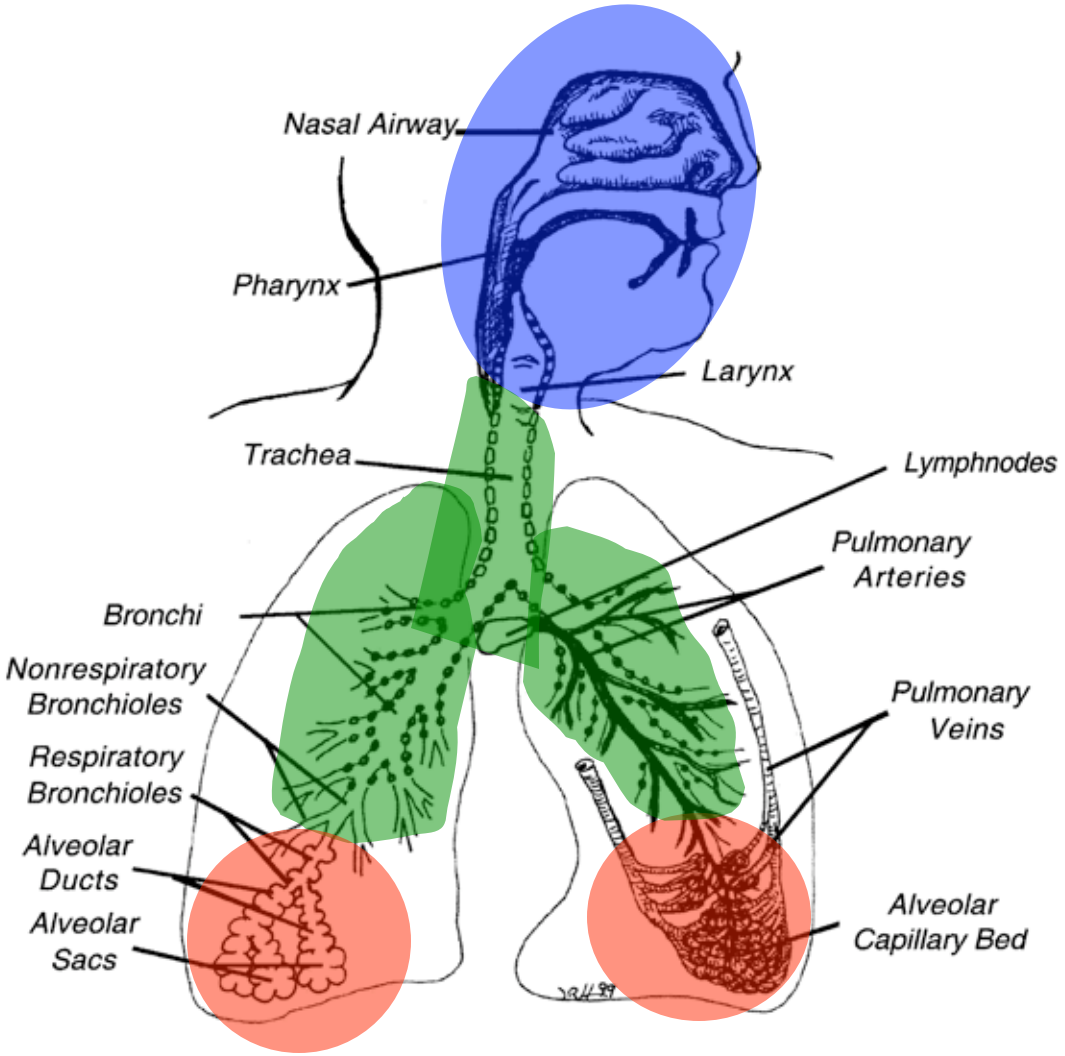
Skin penetration

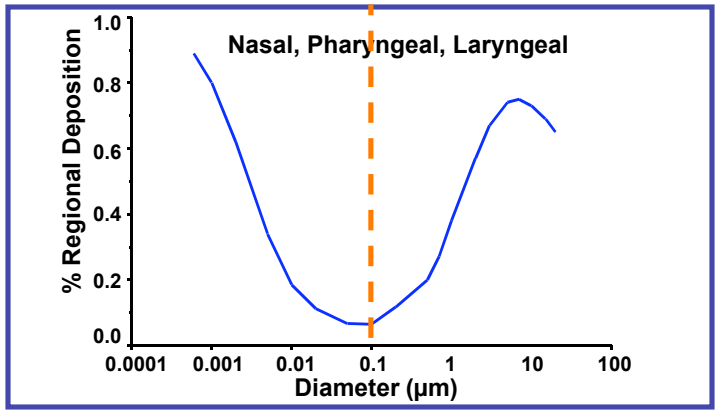


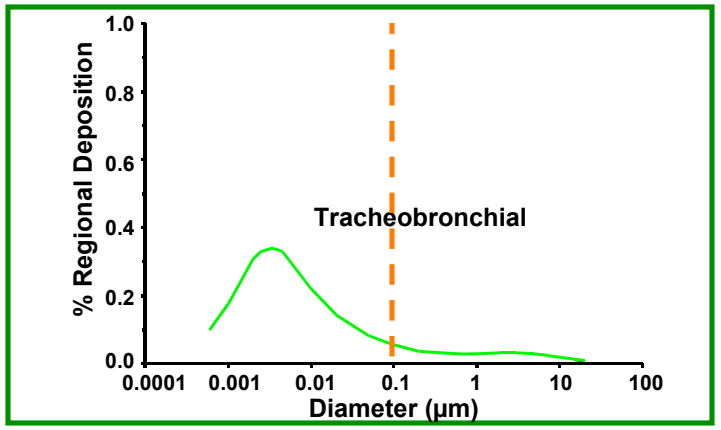
Monteiro, EMS, 2007

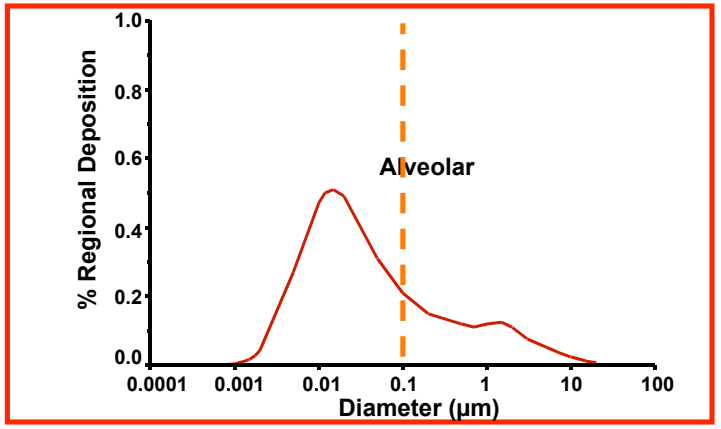
Scale bars 50 um

Fractional Deposition of Inhaled Particles in the Human Respiratory Tract
(ICRP Model, 1994; Nose-breathing)









Oberdörster et al, 2005; Drawing courtesy of J.Harkema

Toxic Warnings

- 1997 - *Titanium dioxide/zinc oxide* nanoparticles from sunscreen are found to cause free radicals in skin cells, damaging DNA. (Oxford University and Montreal University) Dunford, Salinaro et al.
- March 2002 – … *engineered nanoparticles* accumulate in the organs of lab animals and are taken up by cells…“ Dr. Mark Wiesner
- March 2003 - ... studies on effects of *nanotubes* on the lungs of rats produced more toxic response than quartz dust.“ „Scientists from DuPont Haskell laboratory present varying but still worrying findings on nanotube toxicity. Nanotubes can be highly toxic." - Dr. Robert Hunter (NASA researcher)
- March 2003 - Dr. Howard: the smaller the particle, the higher its likely toxicity and that *nanoparticles* have various routes into the body and across membranes such as the blood brain barrier. ETC Group
- July 2003 - Nature reports on work by CBEN scientist Mason Tomson that shows *buckyballs* can travel unhindered through the soil. "Unpublished studies by the team show that the nanoparticles could easily be absorbed by earthworms, possibly allowing them to move up the food-chain and reach humans" - Dr. Vicki Colvin, the Center's director.

Toxic Warnings

- January 2004 - Dr. Günter Oberdörster: *nanoparticles* are able to move easily from the nasal passageway to the brain.
- January 2004 - Nanosafety researchers from University of Leuven, Belgium in Nature: *nanoparticles* will require new toxicity tests: "We consider that producers of nanomaterials have a duty to provide relevant toxicity test results for any new material, according to prevailing international guidelines on risk assessment. Peter H. M. Hoet, Abderrrahim Nemmar and Benoit Nemery, University of Belgium(14)
- January 2004 - Nanotox 2004: Dr. Vyvyan Howard presents initial findings that *gold nanoparticles* can move across the placenta from mother to fetus.
- February 2004 - Scientists at University of California, San Diego discover that *cadmium selenide nanoparticles* (quantum dots) can break down in the human body potentially causing cadmium poisoning. "This is probably something the [research] community doesn't want to hear." - Mike Sailor, UC San Diego.(16)
- March 2004 - Dr. Eva Oberdörster: *buckyballs (fullerenes)* cause brain damage in juvenile fish along with changes in gene function. "Given the rapid onset of brain damage, it is important to further test and assess the risks and benefits of this new technology before use becomes even more widespread." - Dr. Eva Oberdörster.

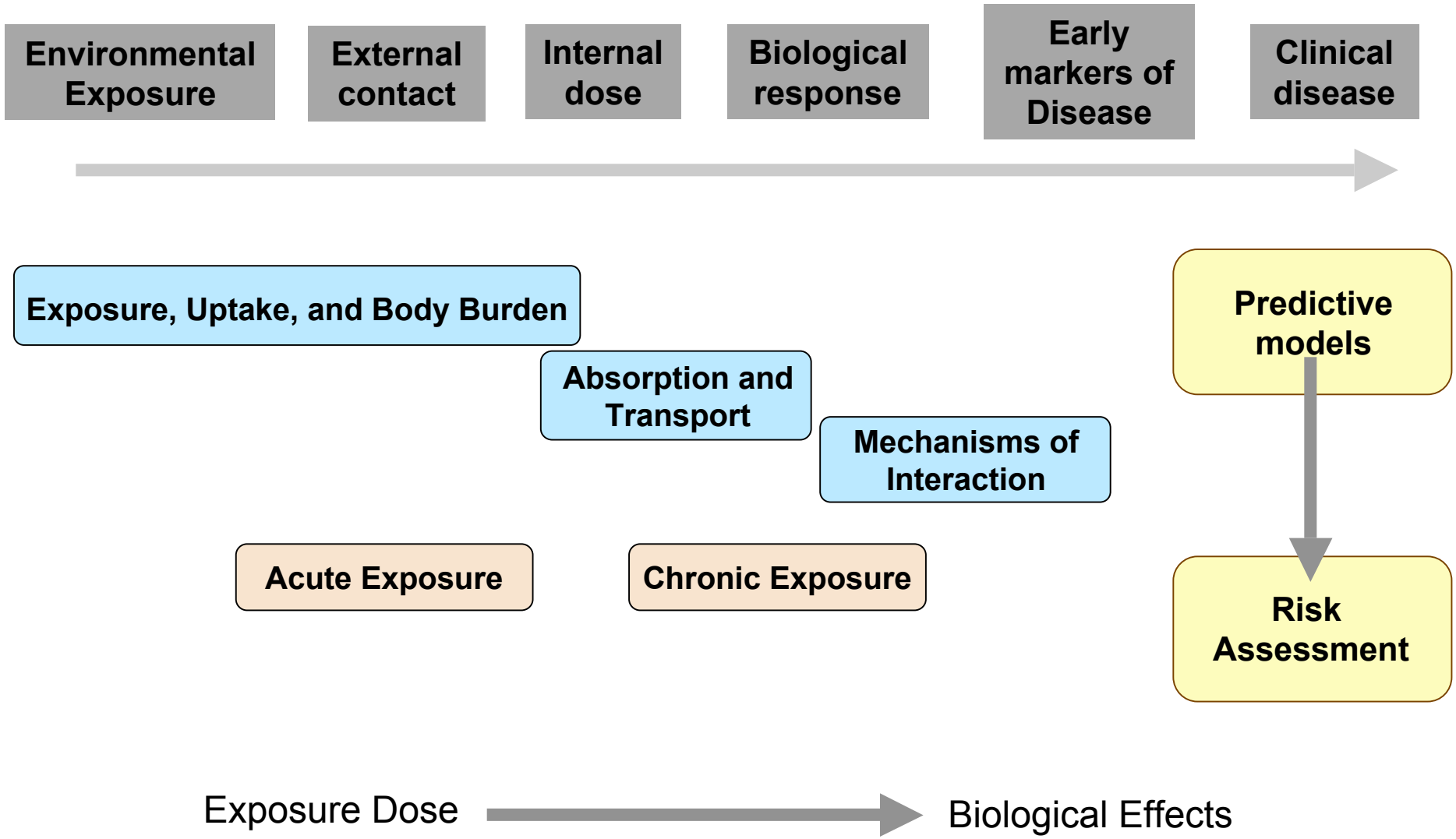
Safety Concerns

- **As particle size gets smaller, there may be size-specific effects on activity, such as:**
  - **Will nanoparticles gain access to tissues and cells that normally would be bypassed by larger particles?**
  - **Once nanoparticles enter tissues, how long do they remain there and how are they cleared?**
  - **If nanoparticles enter cells, what effects do they have on cellular and tissue functions? Might there be different effects in different cells types?**

Safety Concerns (Cont'd)

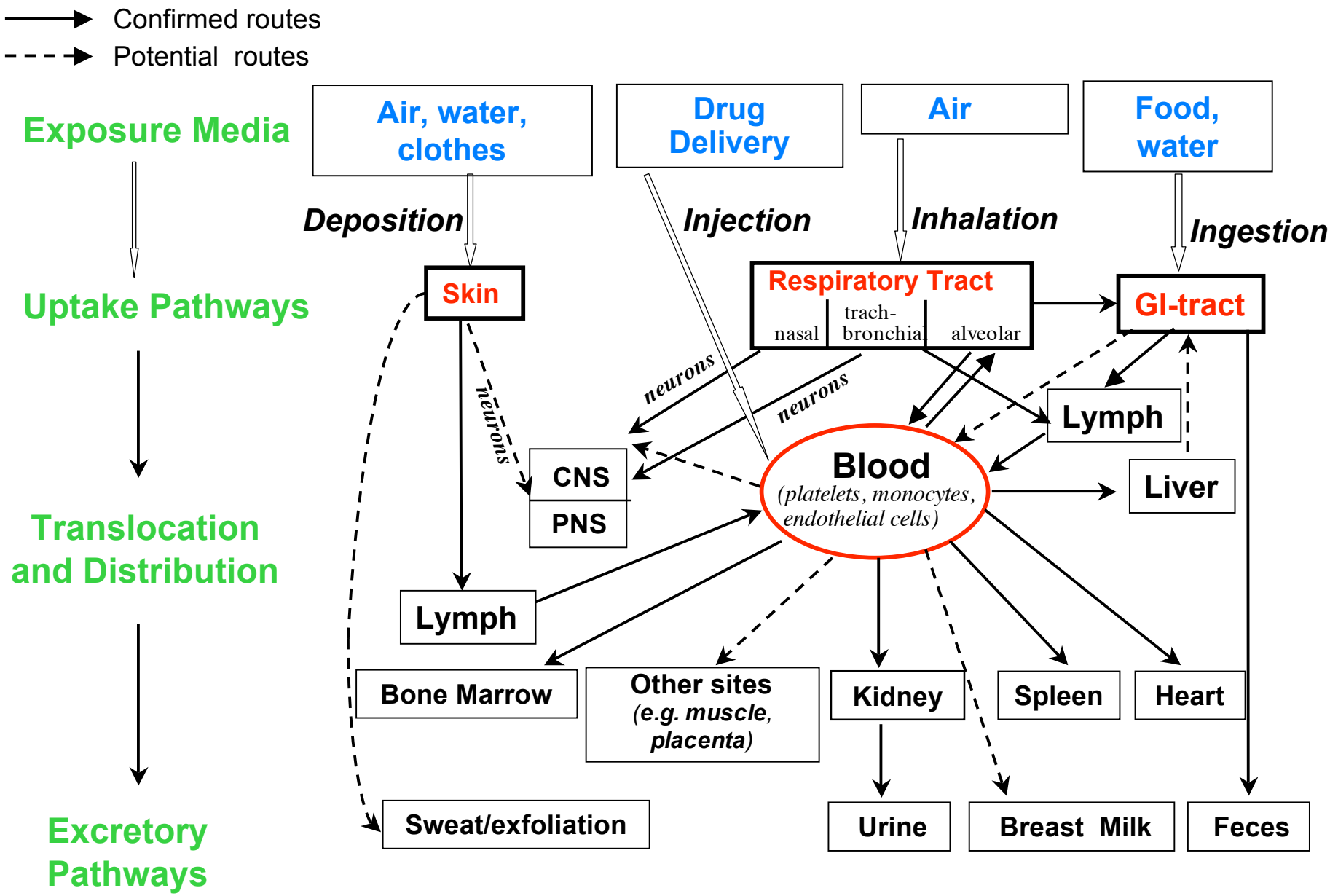
- What are the differences in the profile of nanoparticles versus larger particles?
- What preclinical screening tests would be useful to identify potential risks (in vitro or in vivo)?
- Can new technologies such as "omics" help identify potential toxicities and how can these methodologies complement current testing requirements?
- Can nanoparticles gain access to the systemic circulation from dermal exposure? If nanoparticles enter skin cells, is there an effect on cellular functions? This would be relevant to drugs and cosmetics.

Bio-Interaction of Engineered Nanomaterials



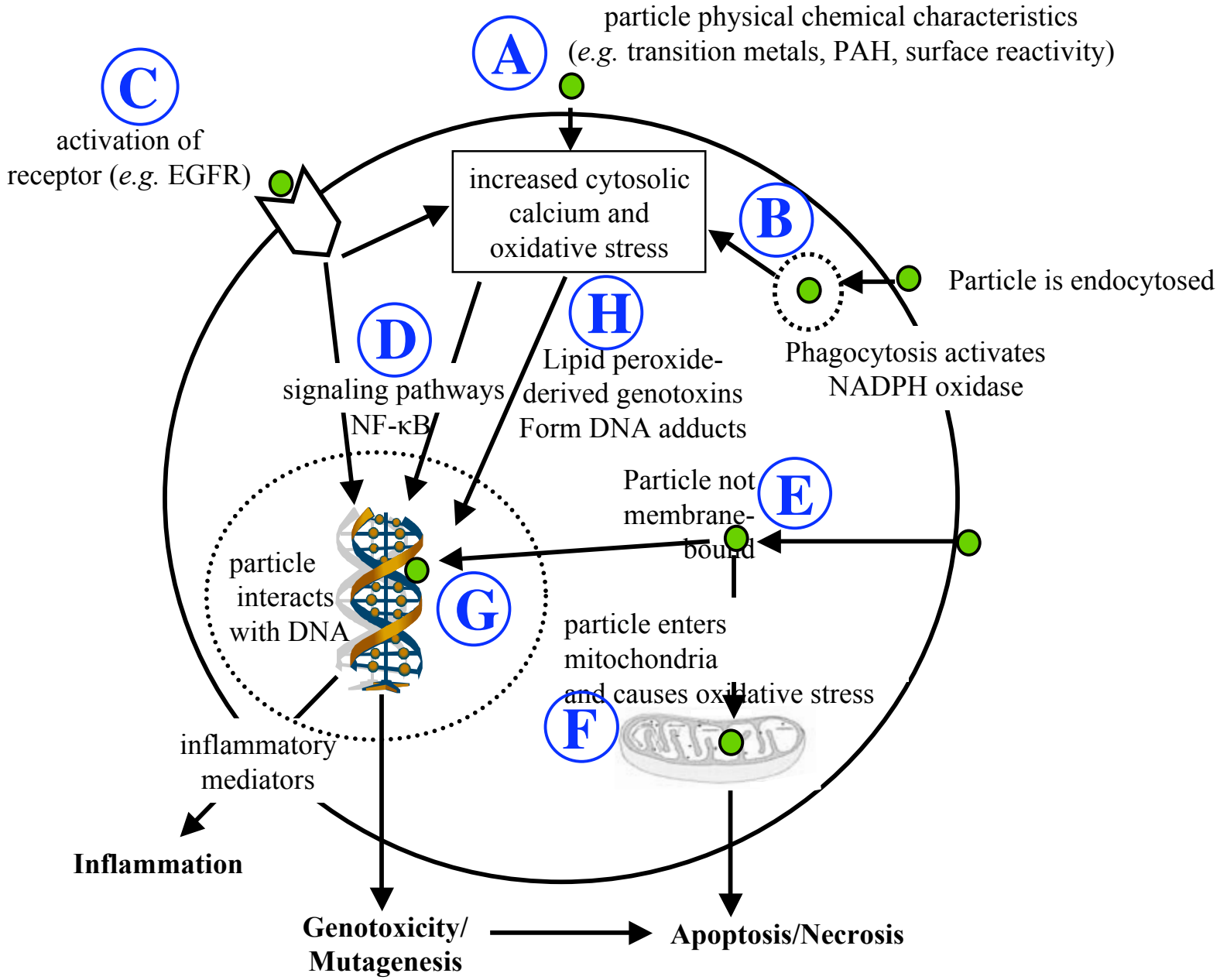
Courtesy, Tinkle

Exposure and Biokinetics of Nanosized Particles



(Oberdorster et all, 2005)

Nanoparticle – Cell Interactions and Oxidative Stress



Oberdorster, EMS 2007

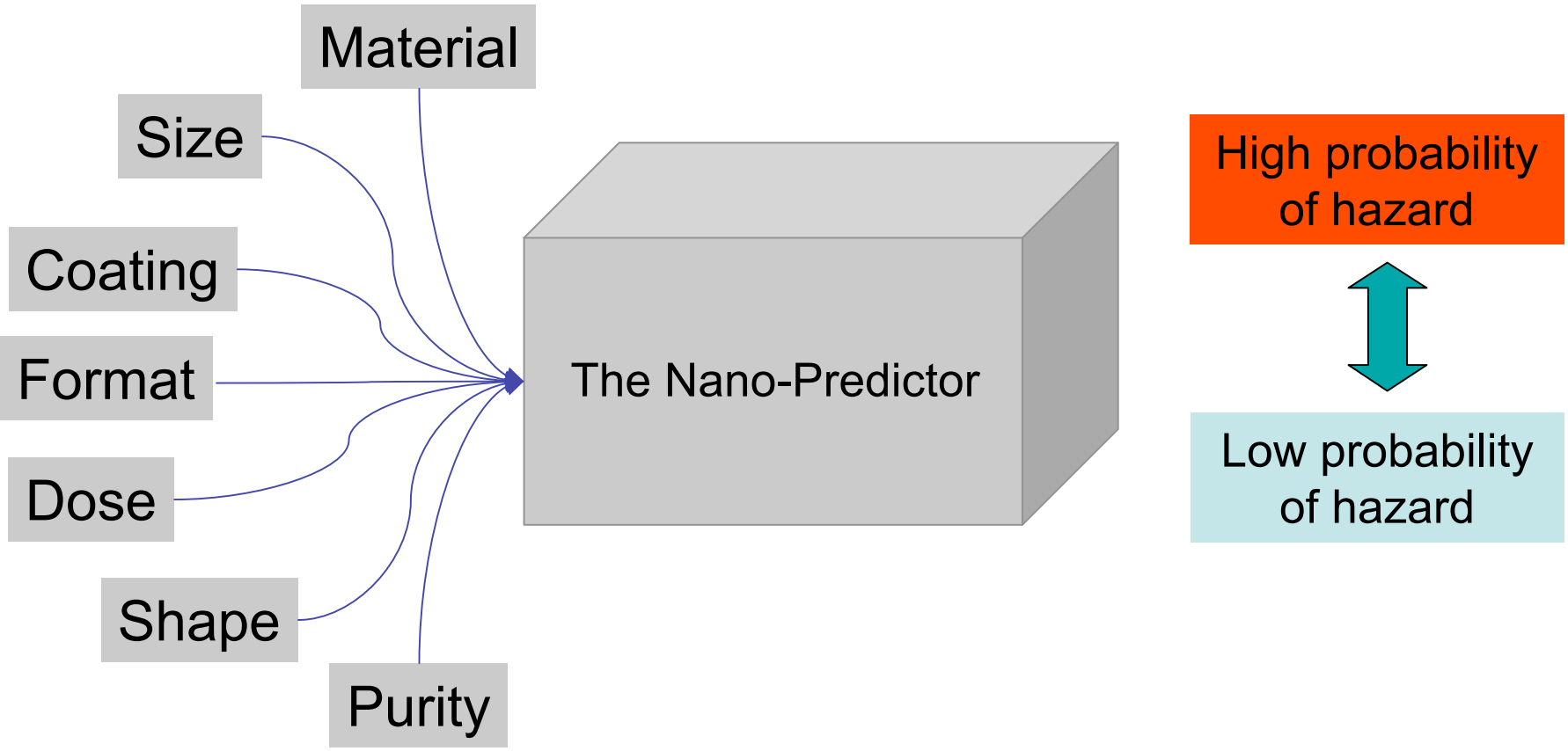
Characterization Concerns

- What are the forms in which particles are presented to host, cells and organelles?
- What are the critical physical and chemical properties including residual solvents, processing variables, impurities and excipients?
- What are the standard tools used for this characterization?
- What are validated assays to detect and quantify nanoparticles in tissues, medical products, foods and processing equipment?
- How do physical characteristics impact product quality and performance?
- How do we determine long and short-term stability of nanomaterials?

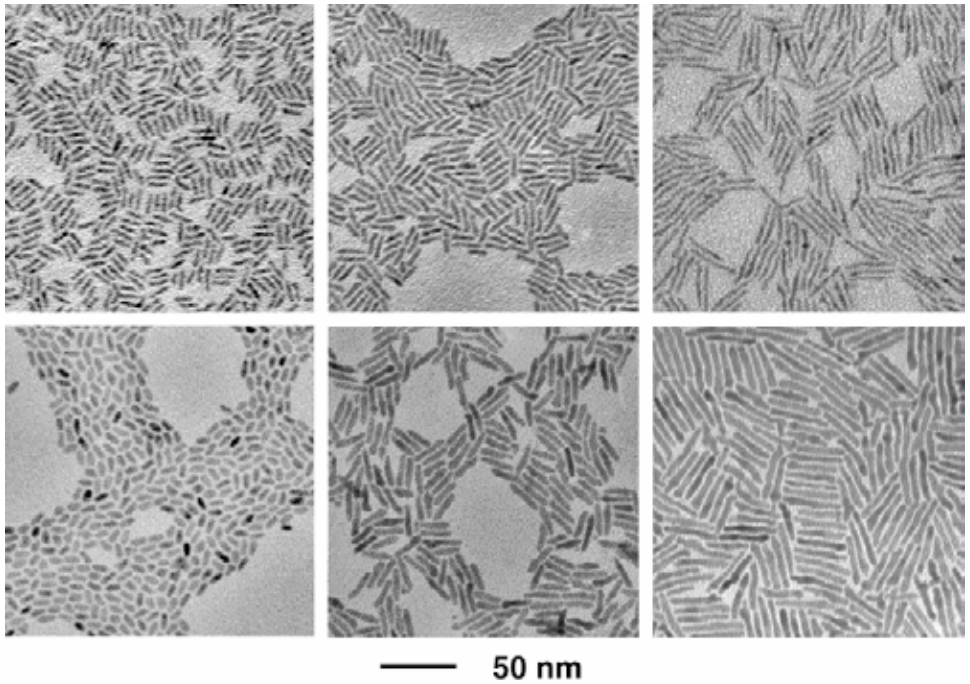
Environmental Concerns

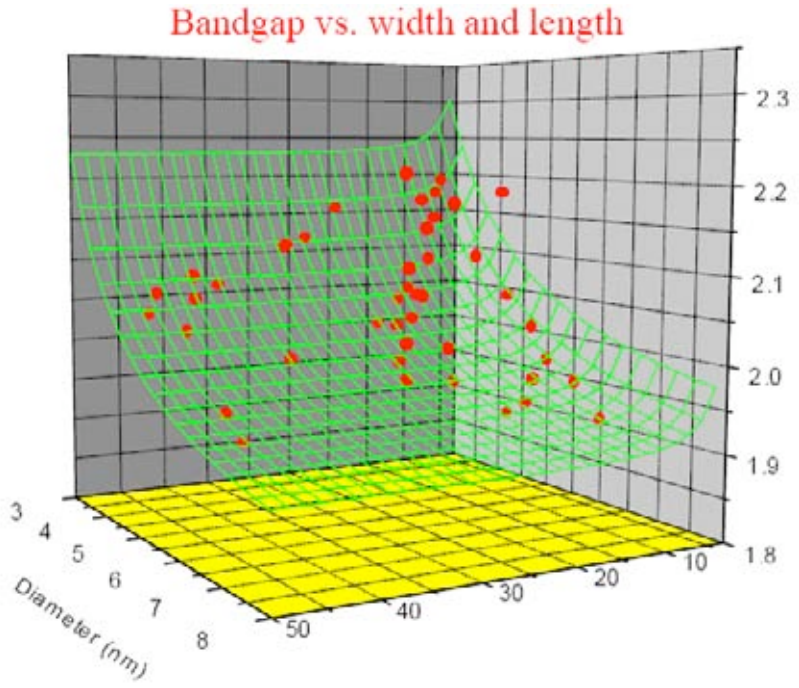
- Can nanoparticles be released into the environment following human and animal use?
- What methodologies would identify the nature, and quantify the extent, of nanoparticle release in the environment?
- What might be the environmental impact on other species (animals, fish, plants, microorganisms)?

Nanosafety management



Nanomaterial chemical/physical matrix

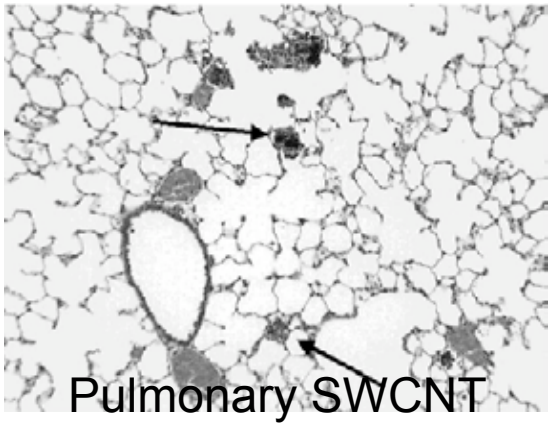


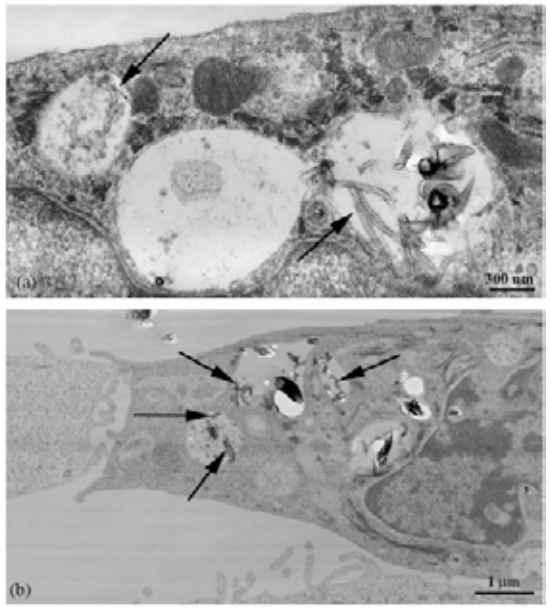


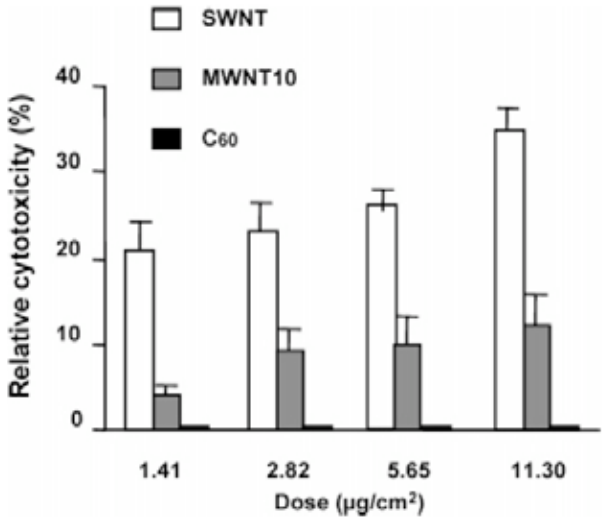
Independent control of length and diameter

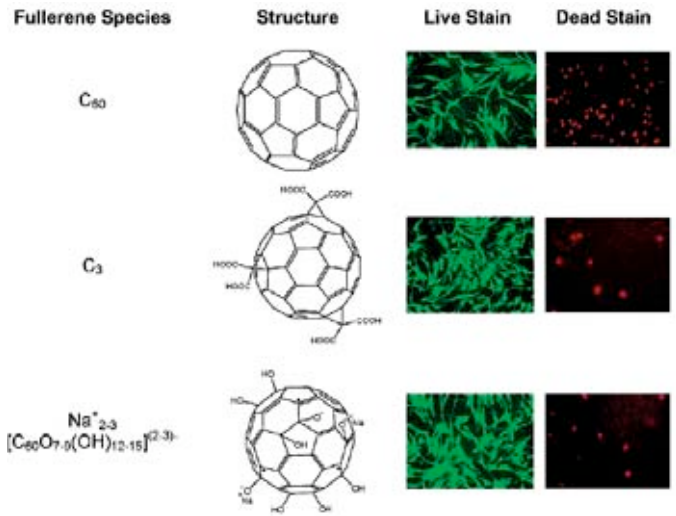
Li, L. S., J. T. Hu, W. D. Yang and A. P. Alivisatos (2001). "Band gap variation of size and shape-controlled colloidal CdSe quantum rods." Nano Letters **1**(7): 349-351.

Nanotoxicity characterization



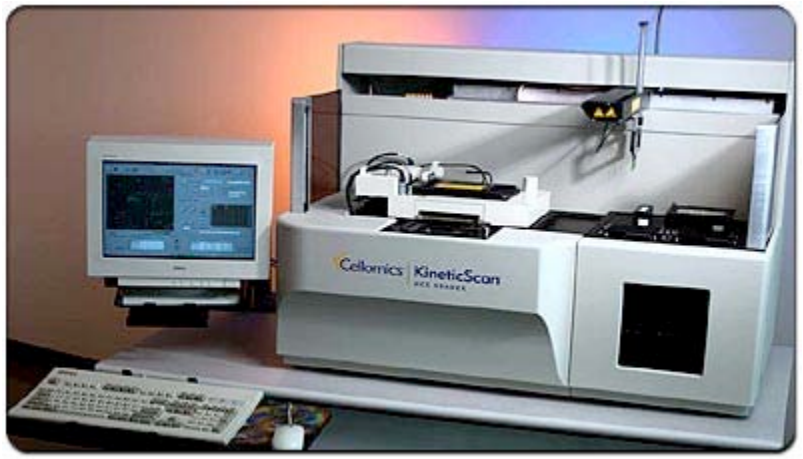




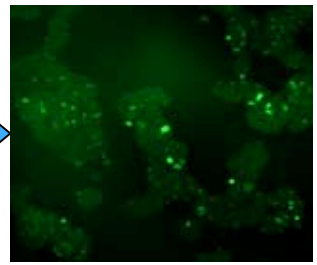


Warheit, D.B. et al. TOXICOLOGICAL SCIENCES 77, 117–125 (2004); Jia, G. et al. Environ. Sci. Technol. 2005, 39, 1378-1383; Monteiro-Riviere, N.A. Toxicology Letters 155 (2005) 377–384; Sayes, C.M. Nano Letters 4, 1881

High Content Analysis of Pathway Activation/Interference



Relocalization of $p27^{kip}$ to nucleus after treatment with herceptin



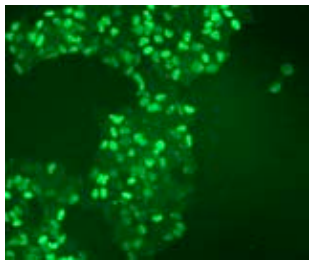


Image analysis performed on thousands of cells to ascertain response

Treat and analyze with Cellomics ArrayScan

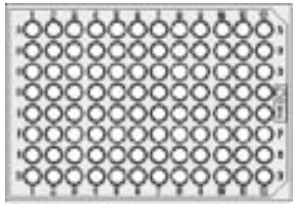
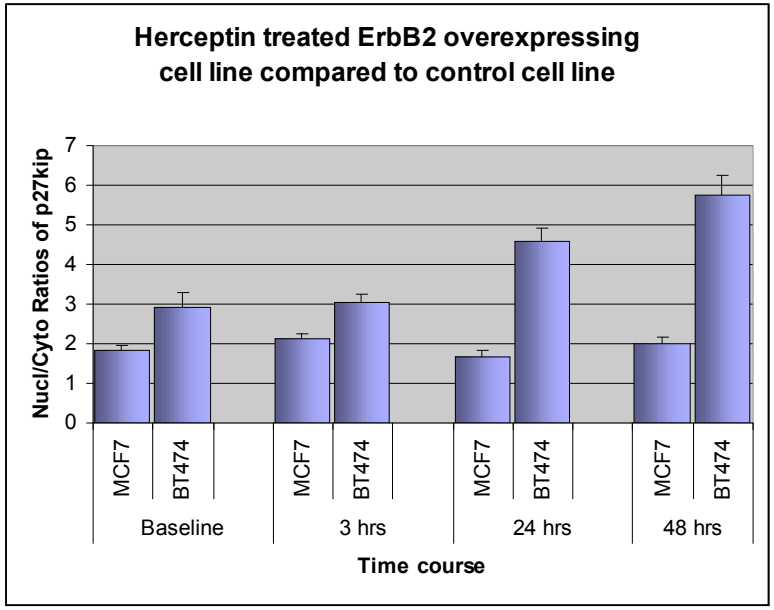


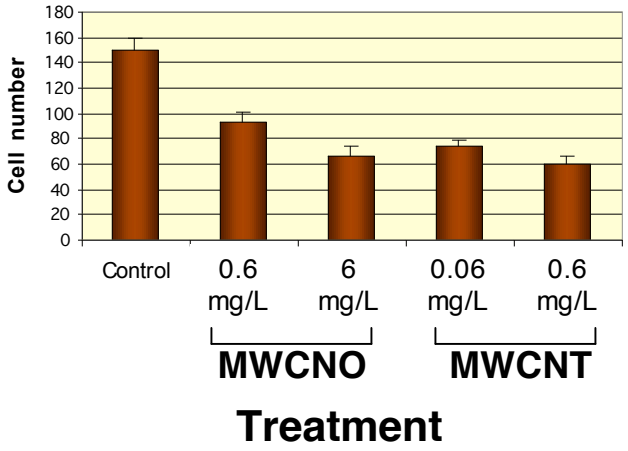
Plate cells on 96-well plate



Apoptosis & Necrosis

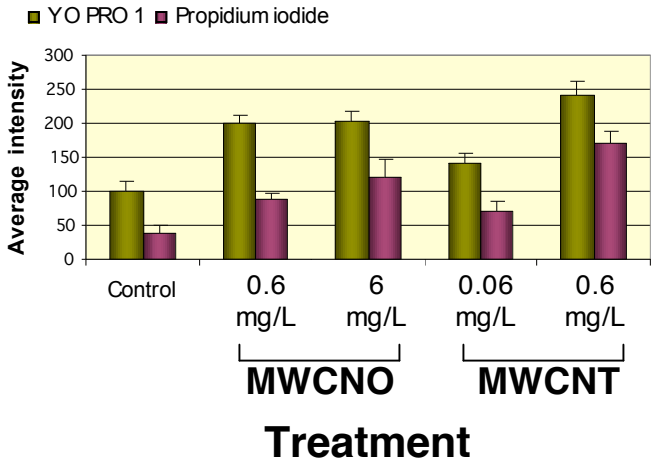
A

Number of Cells

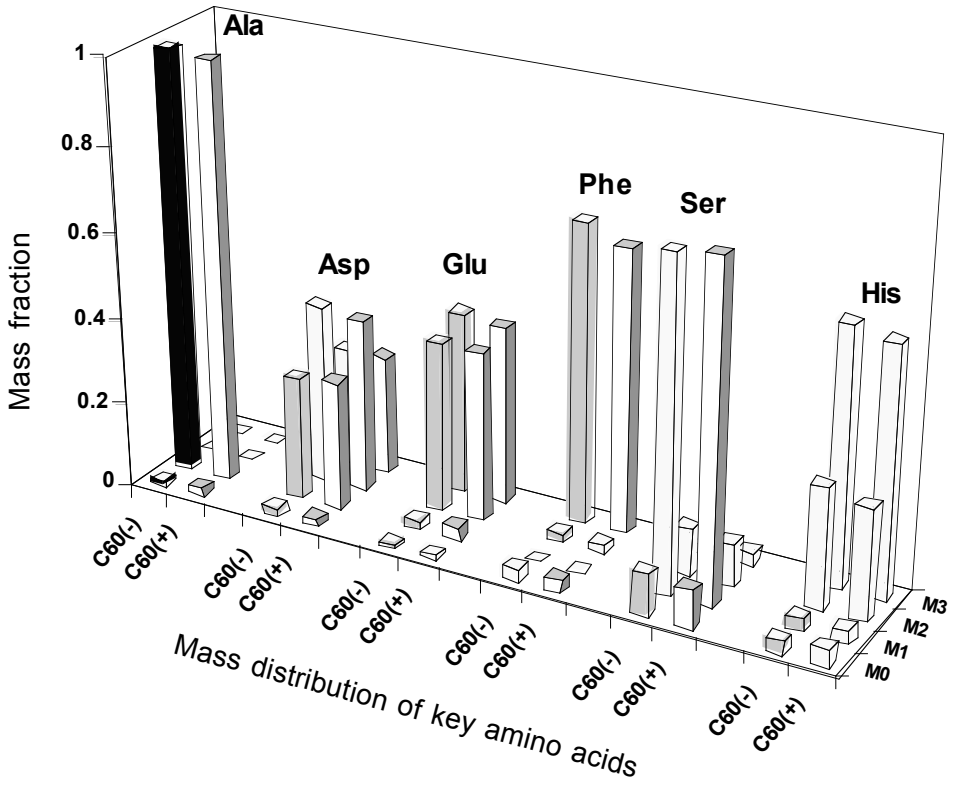


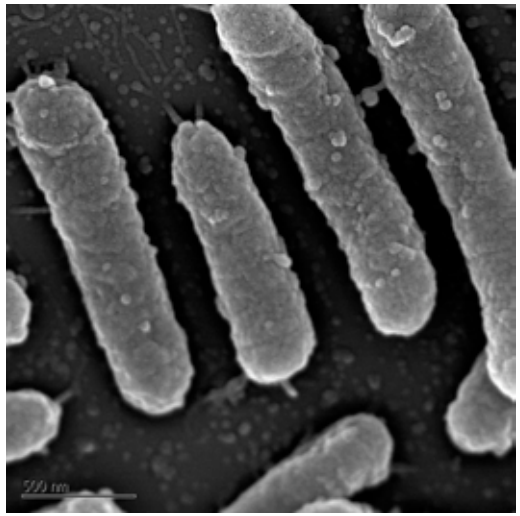
B

Apoptosis and Necrosis

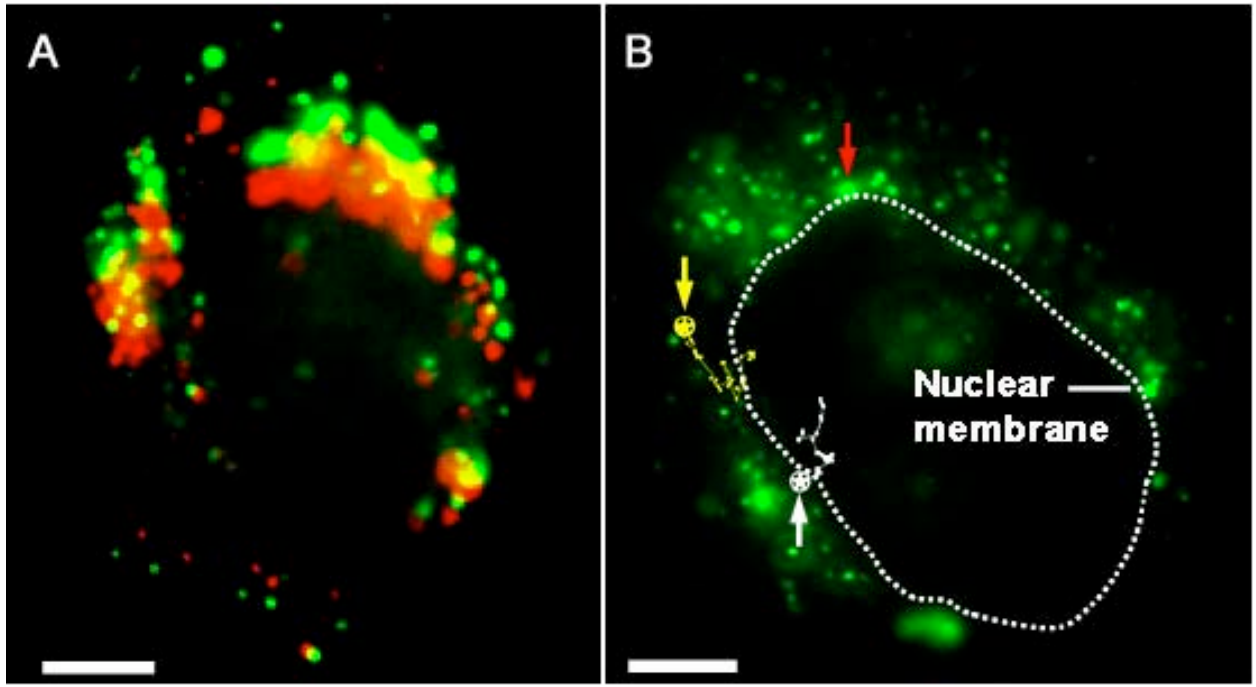


Metabolomic profiling

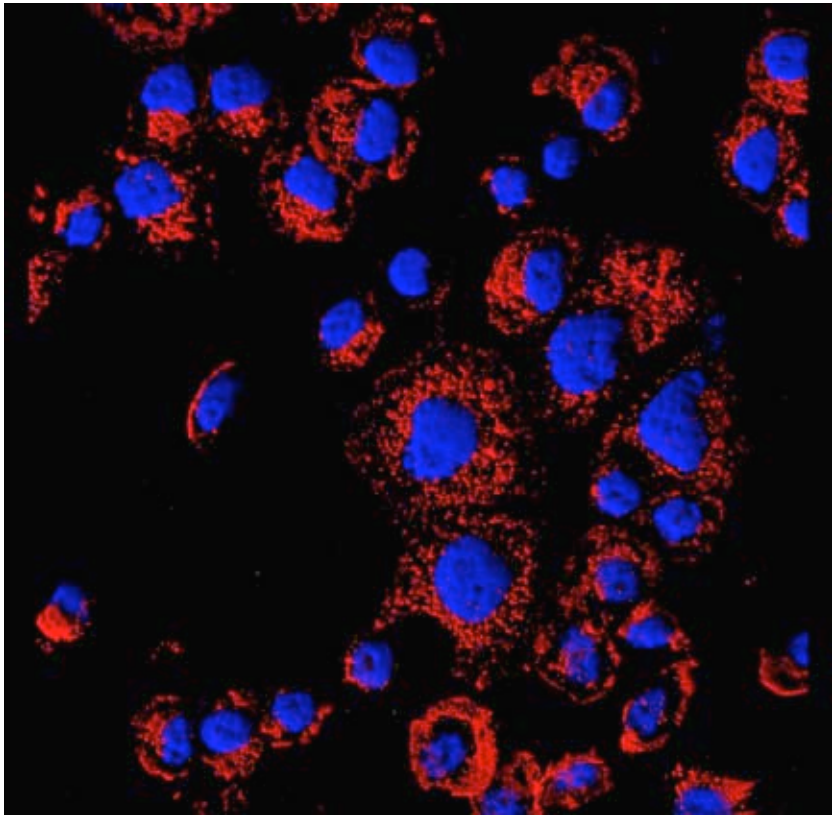




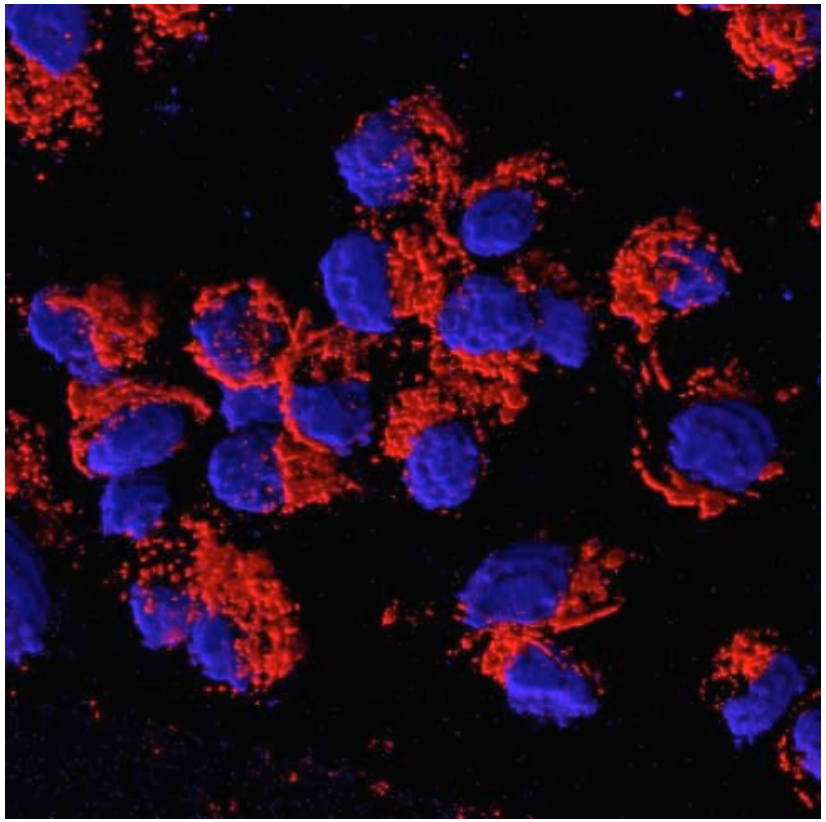
Intracellular movement overtime



Drug delivery nanoparticle tracking

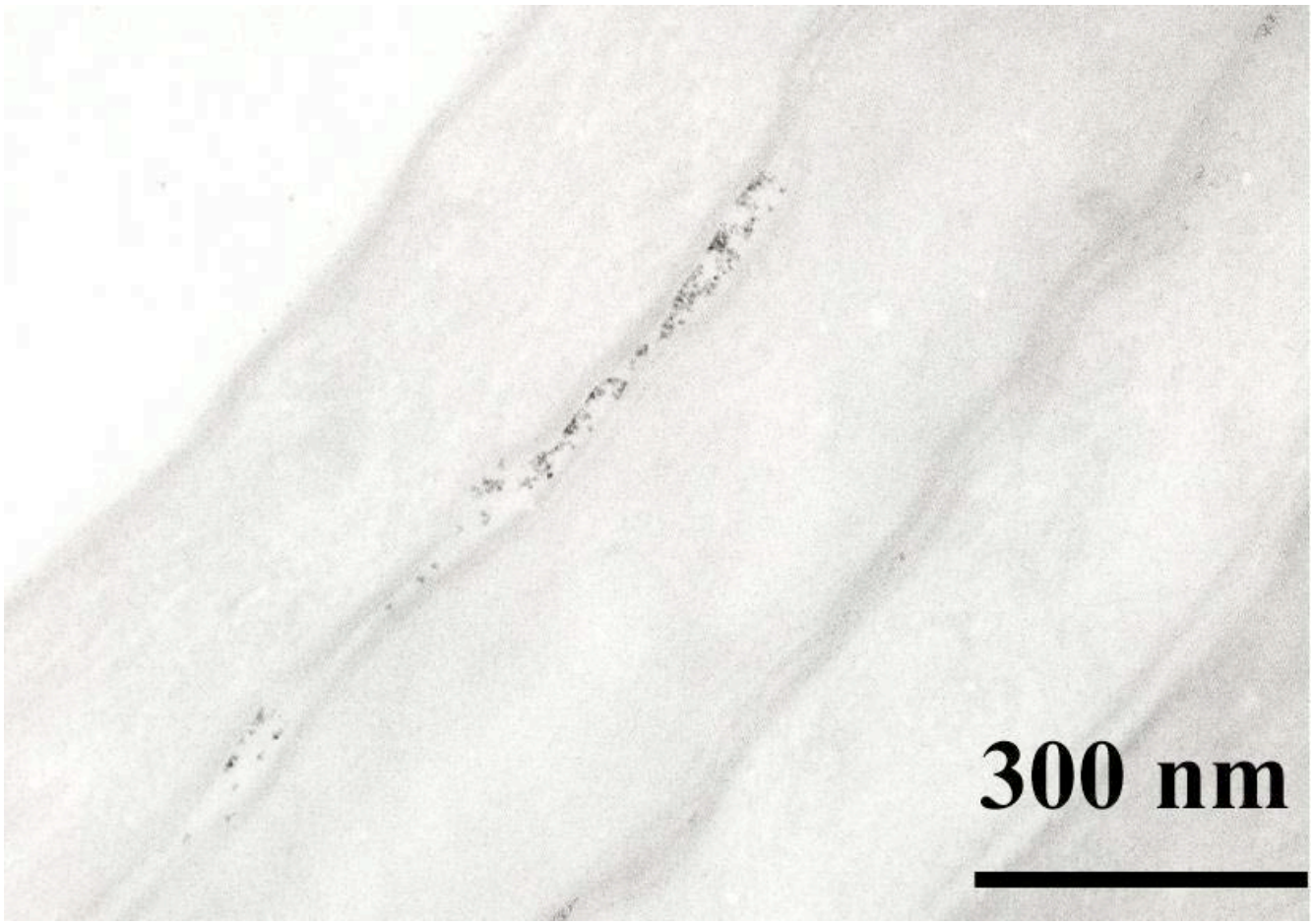


Obtained by a 40× / NA 1.3 oil objective
X: 230 mm, Y: 230 mm, Z: 20 mm
Ex: 800 nm × 2
Blue: DAPI (BP 390-465 nm)
Red: QDs (BP 565-615 nm)

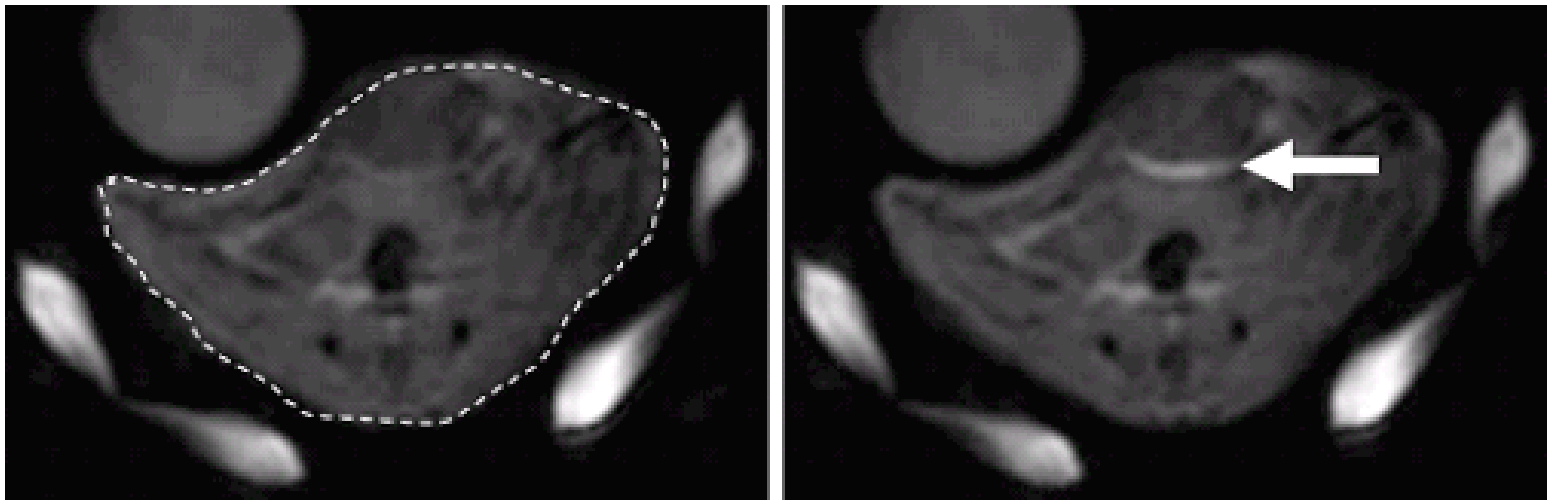


Obtained by a 63× / NA 1.4 oil objective
X: 138 mm, Y: 138 mm, Z: 30 mm
Ex: 800 nm × 2
Blue: DAPI (BP 390-465 nm)
Red: QDs (BP 565-615 nm)

Nanoparticle penetration



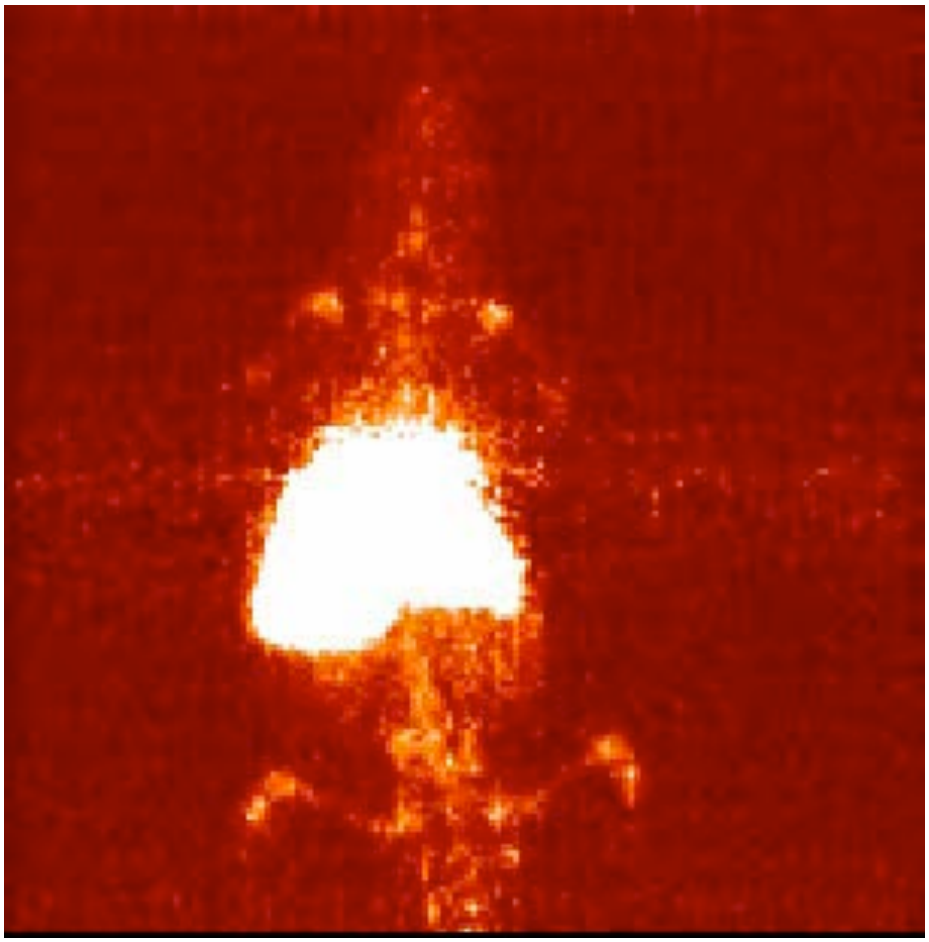
Renal clearance



`Pre-injection

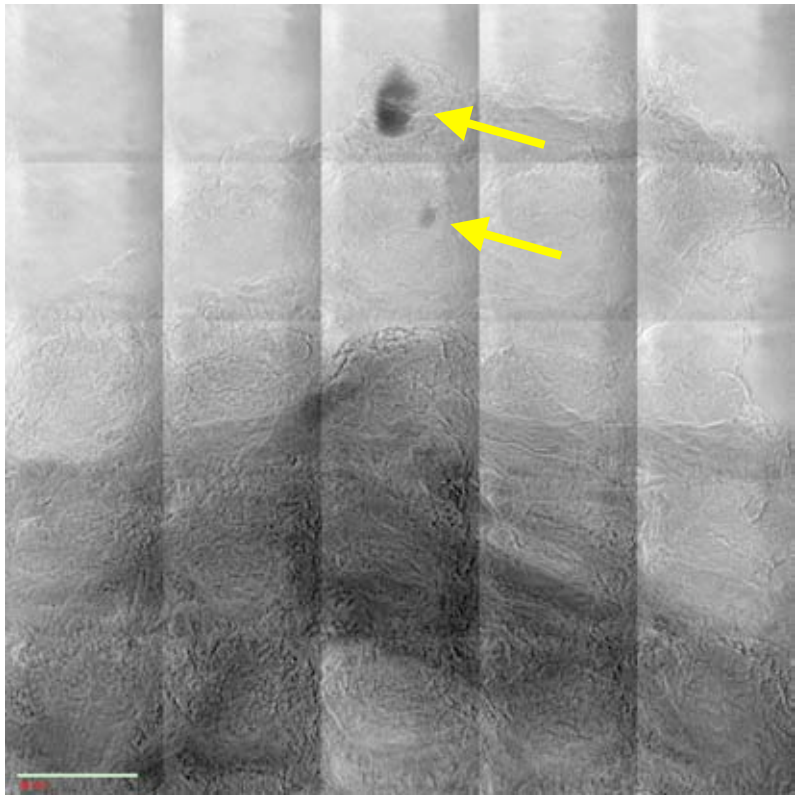
Post-injection

Nanoparticle in vivo PET imaging

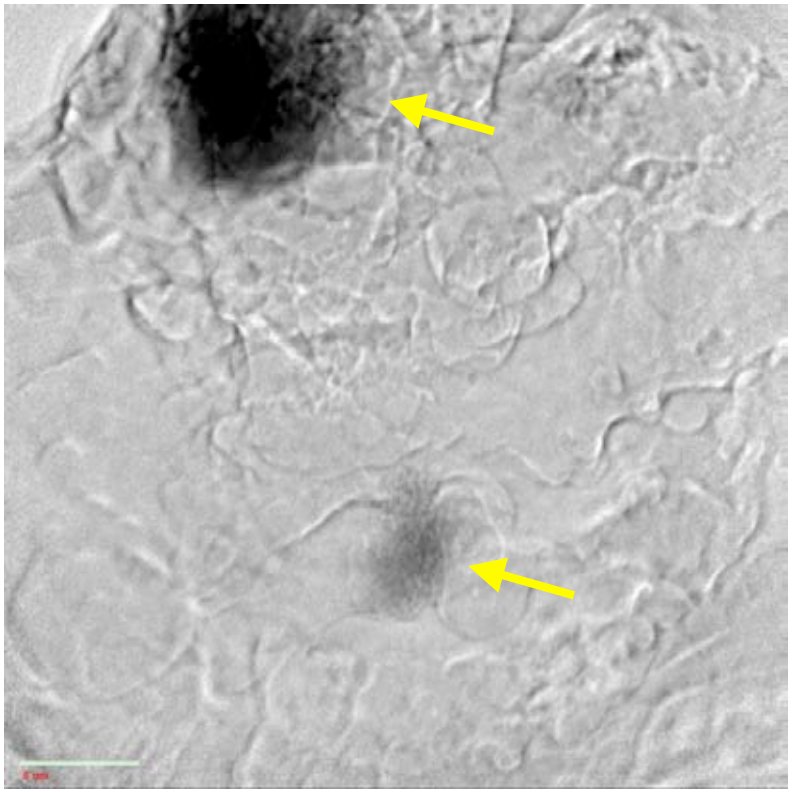


MIP image of biodistribution of ^{64}Cu-quantum dots

Transmission X-ray Microscopy



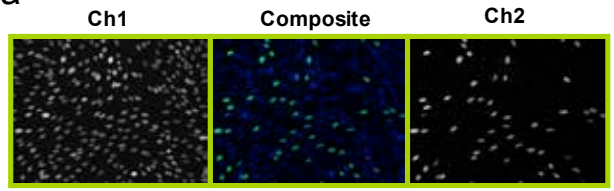
Scale bar
25 mm



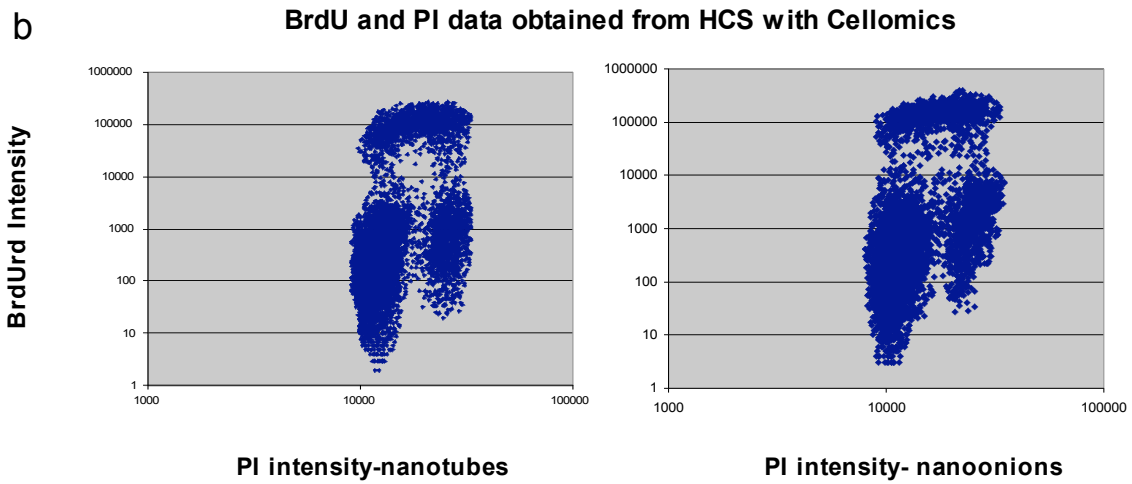
Scale bar
5 mm

Cell cycle perturbation

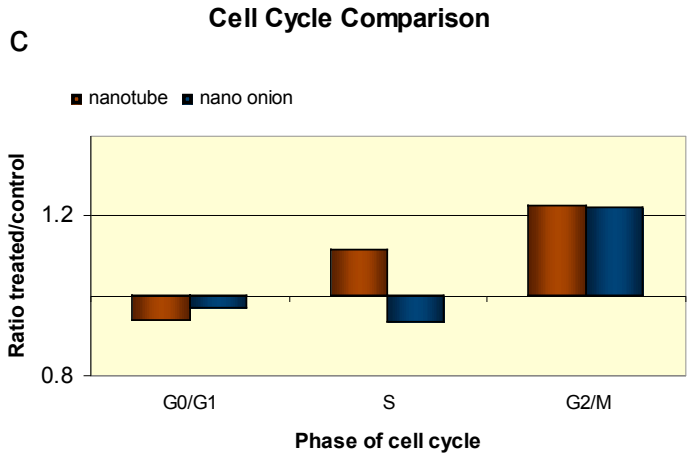
a



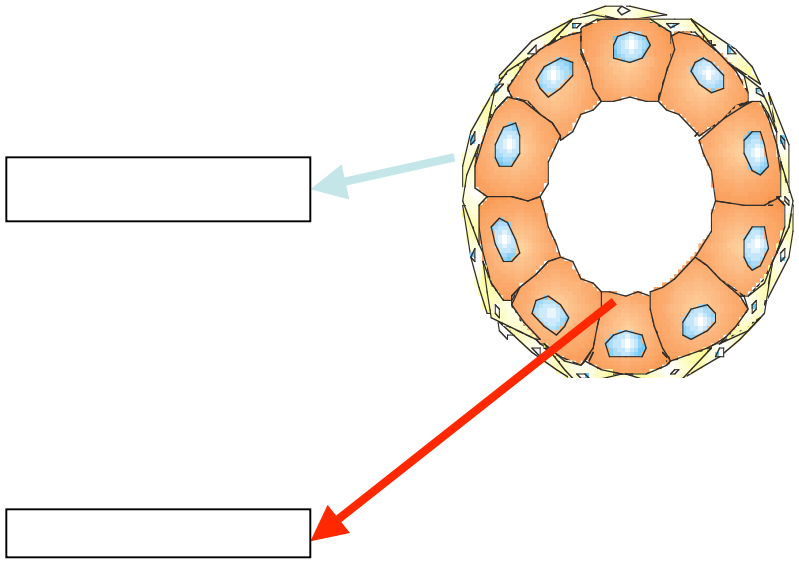
b

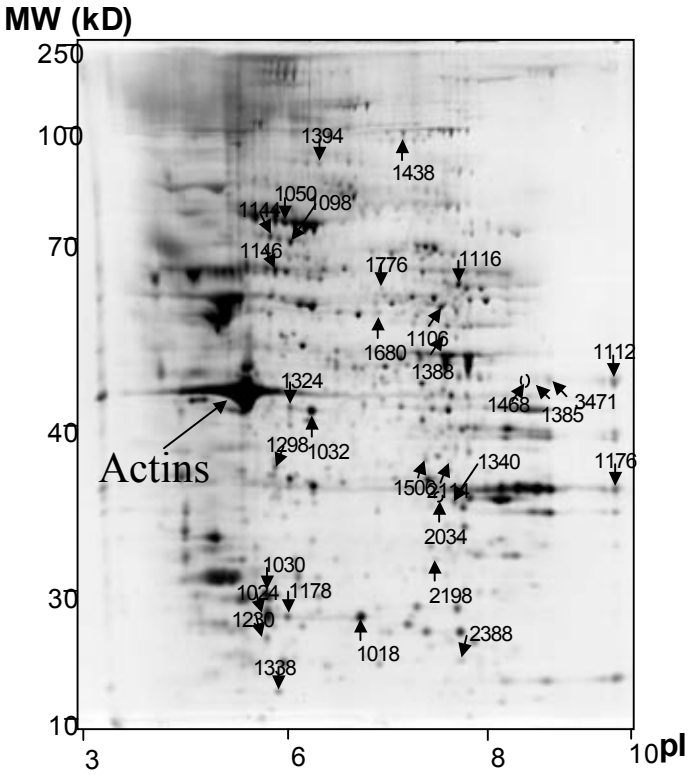


c

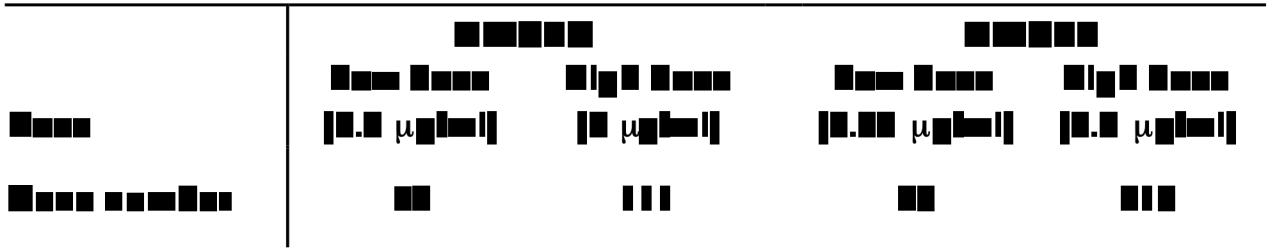


Molecular profiling - signature information of biological systems

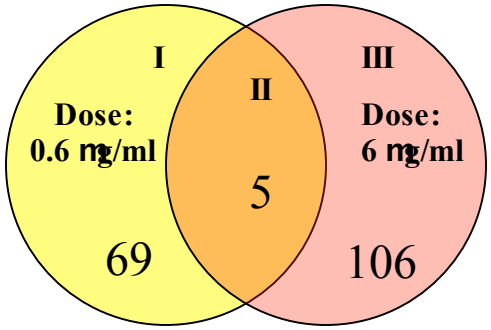




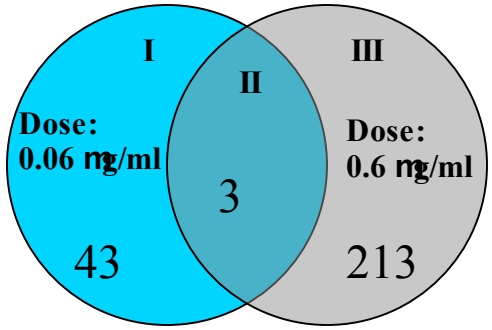
A



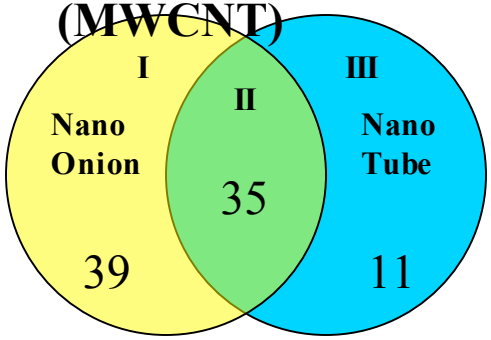
B


Carbon Nano-onion
I
II
III
Dose:
0.6 mg/ml
Dose:
6 mg/ml
5
69
106

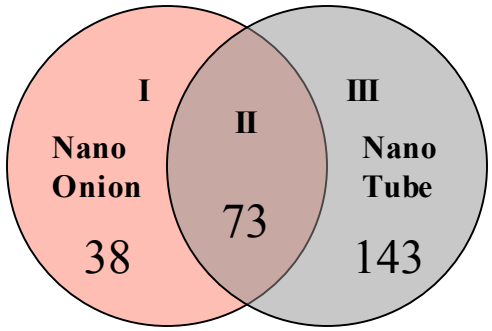
C


Carbon Nanotube
I
II
III
Dose:
0.06 mg/ml
Dose:
0.6 mg/ml
3
43
213

D


0.6 mg/ml (MWCNO)
vs. 0.06 mg/ml
(MWCNT)
I
II
III
Nano
Onion
Nano
Tube
35
39
11

E


6 mg/ml (MWCNO) vs.
0.6 mg/ml (MWCNT)
I
II
III
Nano
Onion
Nano
Tube
73
38
143

Gene Ontology Analysis

CarbonTube 0.06 mg/L

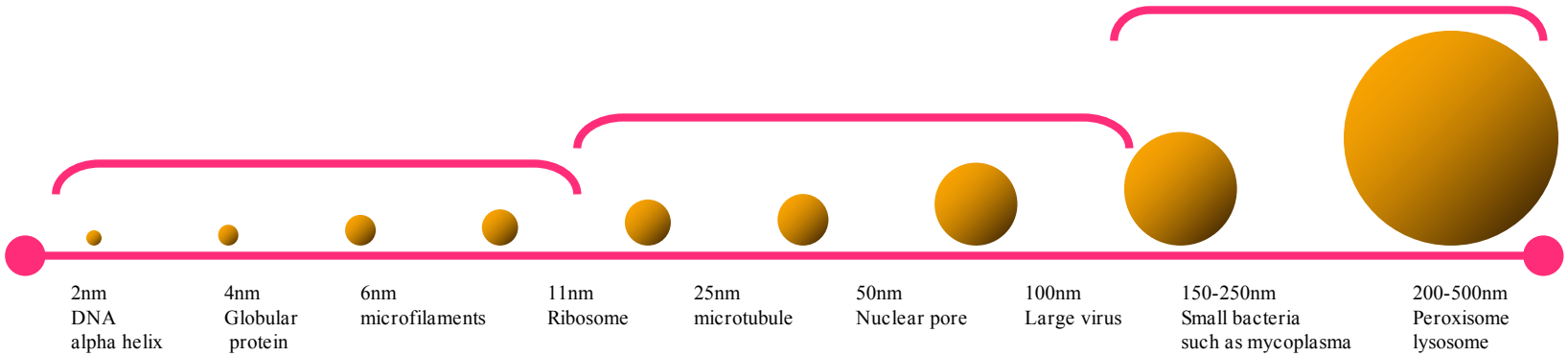
Term	P-Value	Percentage of under expressed	Percentage of over expressed	Percentage of changed
Golgi vesicle transport	0.00070	4.26%	2.13%	6.38%
protein metabolism	0.00200	0.65%	0.18%	0.82%
secretory pathway	0.00490	2.17%	1.09%	3.26%
fatty acid biosynthesis	0.00760	5.71%	0.00%	5.71%
G1/S transition of mitotic cell cycle	0.01350	4.26%	0.00%	4.26%
protein ubiquitination	0.01740	0.68%	1.37%	2.05%
mitotic cell cycle	0.02000	1.95%	0.00%	1.95%
ubiquitin cycle	0.02140	0.70%	0.70%	1.41%
cell homeostasis	0.02280	3.23%	0.00%	3.23%
protein prenylation	0.02620	14.29%	0.00%	14.29%

CarbonTube 0.6 mg/L

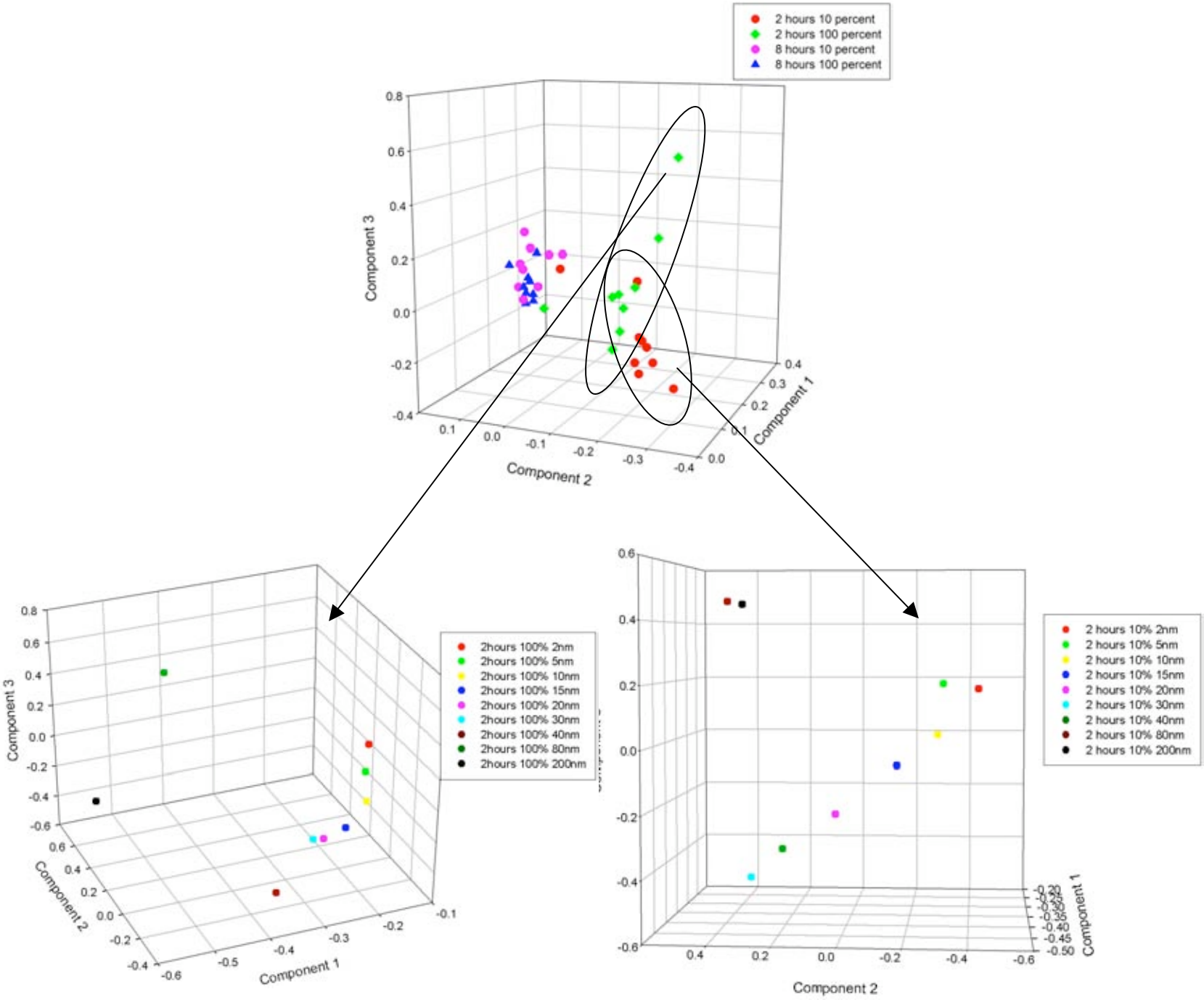
Term	P-Value	Percentage of under expressed	Percentage of over expressed	Percentage of changed
tRNA aminoacylation	0.00000	0.00%	33.33%	33.33%
L-serine metabolism	0.00000	0.00%	50.00%	50.00%
amine metabolism	0.00000	0.00%	6.90%	6.90%
amine transport	0.00000	0.00%	14.63%	14.63%
response to stimulus	0.00000	0.16%	2.86%	3.02%
immune response	0.00000	0.18%	4.50%	4.68%
water-soluble vitamin biosynthesis	0.00240	0.00%	40.00%	40.00%
inflammatory response	0.00340	0.00%	5.06%	5.06%
heterocycle metabolism	0.00620	2.13%	6.38%	8.51%
dicarboxylic acid transport	0.00650	0.00%	25.00%	25.00%

Does Size Matter?

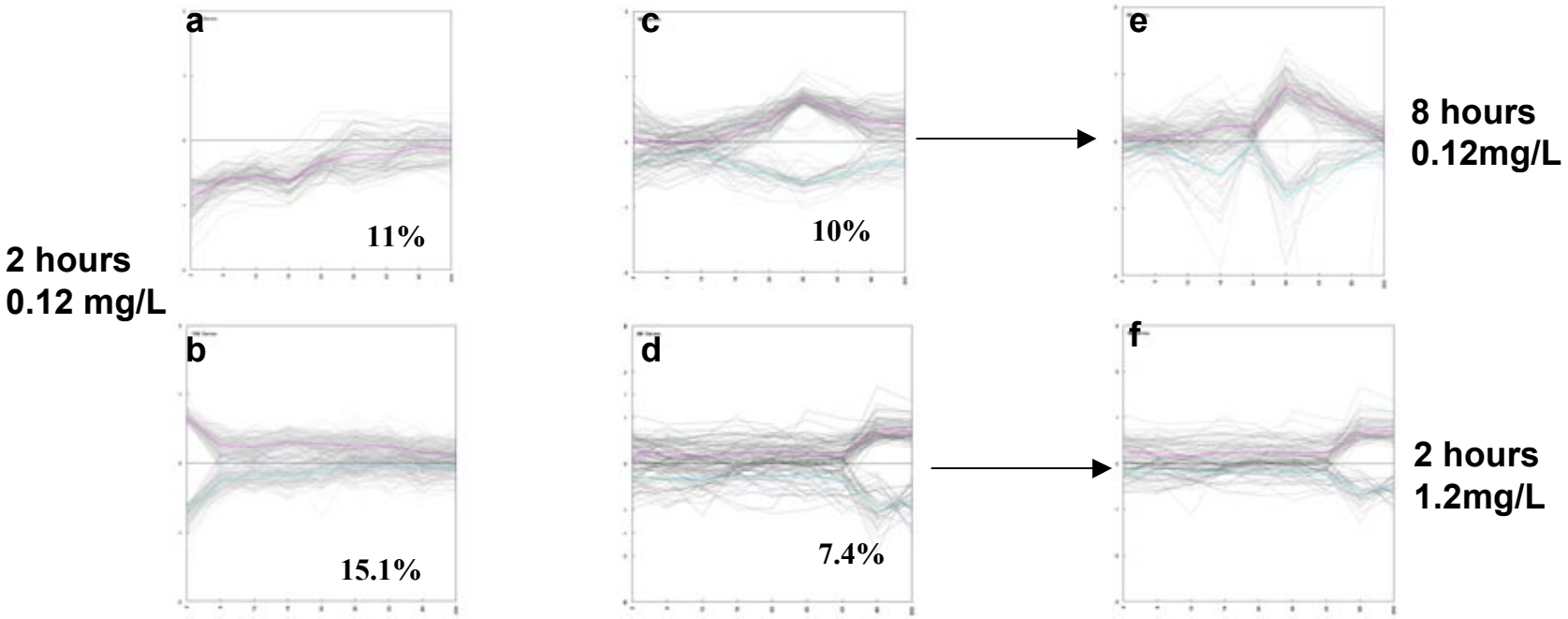
Model system for studying size-dependent effect



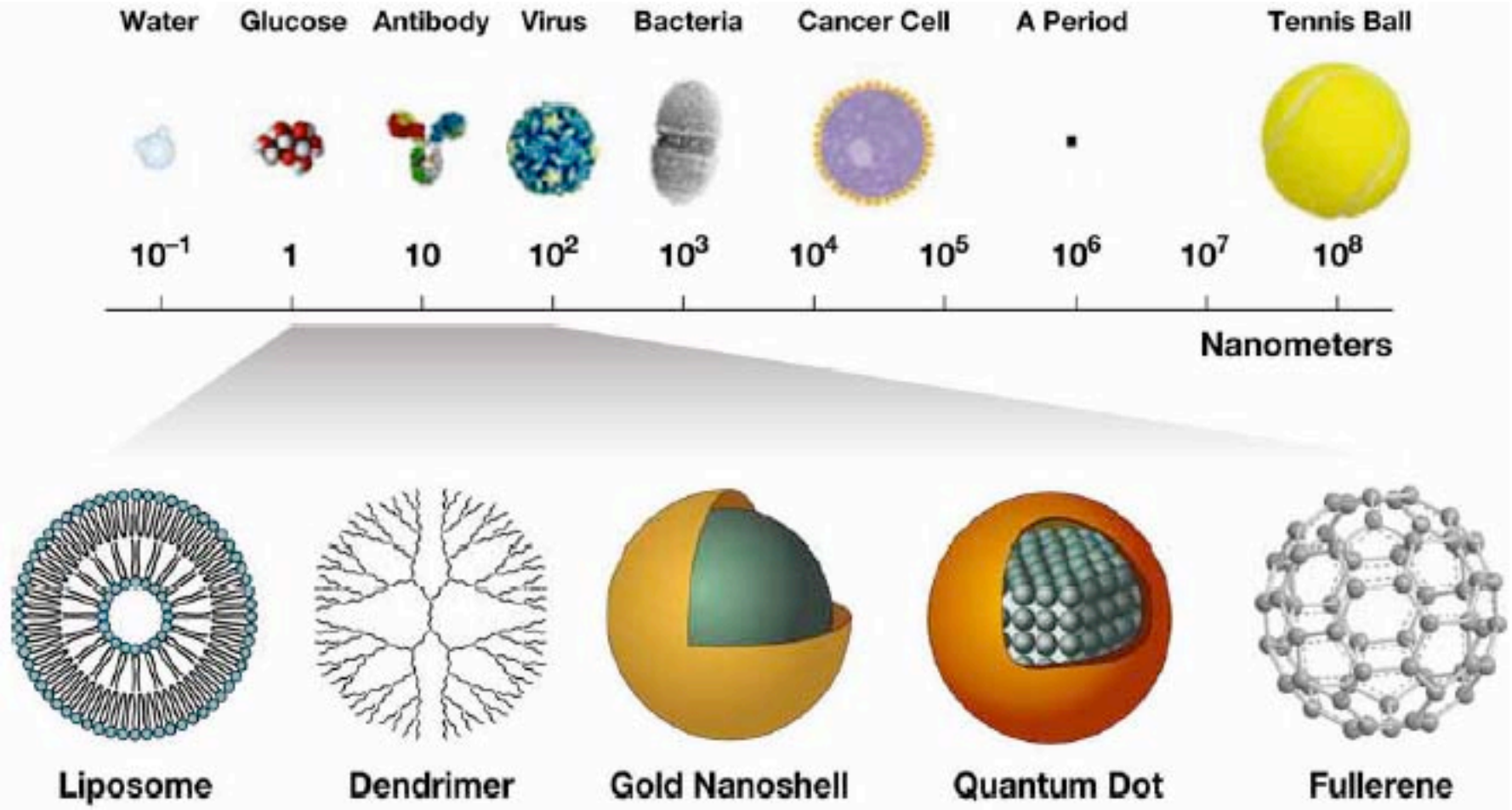
Monodispersed gold nanoparticles interacting with Jurkat lymphocytes


2 hours 10 percent
2 hours 100 percent
8 hours 10 percent
8 hours 100 percent
Component 3
Component 2
Component 1
2hours 100% 2nm
2hours 100% 5nm
2hours 100% 10nm
2hours 100% 15nm
2hours 100% 20nm
2hours 100% 30nm
2hours 100% 40nm
2hours 100% 80nm
2hours 100% 200nm
2 hours 10% 2nm
2 hours 10% 5nm
2 hours 10% 10nm
2 hours 10% 15nm
2 hours 10% 20nm
2 hours 10% 30nm
2 hours 10% 40nm
2 hours 10% 80nm
2 hours 10% 200nm

Size-dependent patterns



Future question: will the physico-chemically related effect be a common phenomenum between different nanoparticles?



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